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Full Name of Author — Nom complet de l'auteur

DAVID NICKOLOS KRISTIE

Date of Birth — Date de naissance	Country of Birth — Lieu de naissance
August 24 / 1953	CANADA

Permanent Address — Résidence fixe

5th Rd. East
RR#1, VINEMOUNT ONTARIO

Title of Thesis — Titre de la thèse

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1979	Dr. M. Spencer

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THE RELATIONSHIP BETWEEN TEMPERATURE AND SECONDARY DORMANCY IN *LACTUCA SATIVA* L.

by

© DAVID NICKOLOS KRISTIE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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IN

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FALL, 1979

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled The Relationship Between Temperature and Secondary Dormancy in Lactuca Sativa L. submitted by David Nickolos Kristie in partial fulfilment of the requirements for the degree of Master of Science.

Mary Spencer
Supervisor

John Hoddinott

Paul R. Gaucher

Date August 21, 1979.

Abstract

The relationship between the upper temperature cut-off point for germination in the light ($UTCP_L$) and the induction of secondary dormancy in lettuce (Lactuca sativa L.) was investigated.

Secondary dormancy was induced in cv. Grand Rapids by prolonged dark incubations at temperatures ranging from 15-35C. Secondary dormancy was also induced in seeds incubated in the dark at 24C in PEG 6000. The length of the dark incubation required to induce a secondary dormancy increased as the incubation temperature was lowered.

The induction of secondary dormancy was related to a decline in the $UTCP_L$. Brief (i.e. less than 24 hours) high temperature incubations reduced the $UTCP_L$ but did not affect germination at low temperatures (ca 15-20C). Prolonged incubations eventually suppressed germination at all temperatures making the seeds secondarily dormant. Prolonged high temperature incubations did not induce a secondary dormancy in cvs. New York or Great Lakes. These treatments reduced the $UTCP_L$ of cv. New York but had little or no effect on the $UTCP_L$ of cv. Great Lakes. Prolonged high temperature incubations reduced the subsequent rate of germination in all three cultivars.

Brief (i.e. less than 24 hours) high temperature incubations also reduced the dark germination of cv. Grand Rapids at all temperatures. In cv. Great Lakes four days of high temperature incubation reduced the rate of dark germination but did not affect the percentage germination.

Secondarily dormant seeds were unable to germinate in response to R, GA_3 or KIN. A combination of R+KIN restored a high level of

germination in secondarily dormant seeds at certain temperatures. R+GA_3 was much less effective.

Transferring heat treated seeds to fresh germination medium promoted germination, suggesting the presence of an inhibitor in the germination medium of heat treated seeds. However, the germination of fresh seeds was not inhibited by the germination medium of heat treated seeds.

The induction of secondary dormancy in cv. Grand Rapids was related to the phytochrome status of the seed. Maintaining Pfr in the seed by repeated exposures to R during high temperature incubations prevented the induction of secondary dormancy but did not prevent a decline in the $UTCP_L$. A single exposure to R at the beginning of the incubation period slowed but did not prevent the induction of secondary dormancy. Incubating seeds in GA_3 mimicked the protective effect of repeated exposures to R.

High temperatures appeared to impose two blocks on the germination of cv. Grand Rapids. High temperatures prevented escape from photoreversibility, and also prevented radicle emergence even after escape had occurred.

Evidence from the present studies suggests that there are qualitative differences between the germination mechanisms of the light sensitive cultivar Grand Rapids and the light insensitive cultivar Great Lakes; and that the induction of secondary dormancy in cv. Grand Rapids is related to an effect on the phytochrome system.

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List of Abbreviations

GA_3	gibberellic acid
KIN	kinetin
ABA	abscisic acid
Pfr	phytochrome - far red absorbing form
Pr	phytochrome - red absorbing form
PEG 6000	polyethylene glycol 6000
UTCP	upper temperature cut-off point
$UTCP_L$	UTCP for germination in the light
$UTCP_D$	UTCP for germination in the dark

1.0 Introduction

1.1 General

Seed germination in many species is strongly temperature dependent. In lettuce (Lactuca sativa L.), high temperatures inhibit germination and reduce seedling emergence in the field (Gray, 1975). Thus, understanding the processes that govern these temperature dependent dormancy phenomena is of practical as well as theoretical interest. The present study was undertaken to clarify the roles that temperature and light play in the imposition and release of secondary dormancy in lettuce.

In a strict botanical sense, the term seed refers to the matured ovule of an angiosperm. However, the structure often termed a seed in the literature is actually a seed enclosed with layers of the fruit coat. In French the terms "diaspore" (dispersal unit) or "semence" (germination unit) adequately describe this structure. Unfortunately, these terms lack a convenient English counterpart. Therefore, in this thesis the term seed will be used to describe the dispersal unit of the fertilized ovule, regardless of its true botanical classification.

The lettuce "seed" is actually an achene, or an indehiscent dry one-seeded fruit. The embryo of the seed consists of an axis and two well-developed cotyledons. The embryo is enclosed by the endosperm, a sac-like organ 2-3 cells thick. Surrounding the endosperm are the remains of a seed coat (testa or integument) fused to the fruit coat or pericarp. In cv. Grand Rapids the fruit coat is heavily pigmented and appears black. In cvs. New York and Great Lakes the fruit coat is white.

The term dormancy implies a state of suspended growth. Numerous definitions of dormancy have been proposed (Koller et al, 1962; Pollack and Toole, 1961; Vegis, 1964; Villiers, 1972). Villiers' (1972) definition has gained wide acceptance and will be used for this thesis. To quote directly:

> . . . dormancy will be reserved to describe the state of arrested development whereby the organ or organism, by virtue of its structure or chemical composition, may possess one or more mechanisms preventing its own germination, while the term quiescence will be used to describe a state of arrested development maintained solely by unfavorable environmental conditions such as inadequate water supply.

Other unfavorable environmental conditions commonly cited include a lack of oxygen and abnormally high or low temperatures. It should be pointed out that in the context of this definition, light is not considered to be an environmental condition. Therefore, seeds unable to germinate because of unsuitable light conditions are considered to be dormant.

The factors that impose seed dormancy and the conditions that release it are extremely diverse. Several attempts have been made to classify seed dormancy according to the mechanisms that prevent germination (Crocker, 1916; Nikolaeva, 1969; 1977; Villiers, 1975; Wareing et al., 1973).

Nikolaeva (1969; 1977) has presented the most comprehensive classification system to date. In this scheme a distinction is made between dormancy imposed solely by the seed coverings (exogenous dormancy) and dormancy imposed by an internal condition of the embryo (endogenous dormancy). There are said to be three "types" of exogenous dormancy, and at least eleven "types" of endogenous dormancy.

Nikolaeva (1977) classifies the dormancy exhibited by lettuce as an endogenous dormancy termed physiological non deep. Physiological non deep dormancy is said to be caused by a double mechanism of decreased activity of the embryo and a restriction of gas exchange by the seed covers. Physiological non deep dormancy is a shallow dormancy and in many species it can be overcome simply by providing a specific temperature or illumination condition. Removing or damaging the seed covers will often promote germination. Non deep dormancy is most pronounced in freshly matured seeds and gradually disappears during after-ripening in dry storage. Non deep dormancy is observed mainly in herbaceous species including cultivated crops such as wheat, barley and sunflower.

1.2 Light and Seed Germination

Seeds whose germination is affected by white light are termed photoblastic or light sensitive. White light may either promote germination (positive photoblastism) or inhibit germination (negative photoblastism). It is now known that the promotive and inhibitory effects of white light result from specific regions of the spectrum. In both positively (Borthwick et al., 1952; Flint and McAllister, 1935; 1937) and negatively (Jones and Bailey, 1956; Yaniv et al., 1967) photoblastic seeds, red light (660nm) promotes germination and far red light (730 nm) inhibits germination. In addition, far red light is able to reverse the promotive effects of red light. Thus in an alternating series of red and far red light treatments, germination is determined by the final light treatment (Borthwick et al., 1952). The different response of positively and negatively photoblastic seeds has

been explained in terms of differing sensitivity to the red and far red regions of the spectrum (Smith, 1975).

The germination of light insensitive or non-photoblastic seeds can also be brought under red/far red control by placing them under conditions of stress, such as imbibition in solutions of high osmotic pressure (Karssen, 1970) or at temperatures above 30C (Mancinelli et al., 1967). These experiments suggest that the germination processes of photoblastic and non-photoblastic seeds are basically the same.

It is now felt that all photoblastic responses are under the control of the photoreceptor phytochrome. The phytochrome molecule is a blue-green chromoprotein (Siegelman and Finer, 1964). The chromophore is thought to be a linear tetrapyrrole (Siegelman et al., 1966).

A mechanism for the reversible photoisomerization of the chromophore has been presented (Rudiger, 1972). Phytochrome exists in two interconvertible forms, termed Pr and Pfr for the red and far red absorbing forms respectively. Absorption of red light by Pr transforms the molecule to the Pfr form and conversely far red light converts Pfr to Pr. Pfr is thought to be the biologically active form of phytochrome. Because of the broad overlapping absorption spectra of the two forms, photoconversion is never complete in either direction (Butler et al., 1964). Red light establishes a photostationary state of 81% Pfr and 19% Pr. Under far red irradiation about 2% of the phytochrome remains in the active Pfr form. Once established, the photostationary state is thought to be independent of irradiance (Schafer, 1975).

Phytochrome can also undergo several other transformations. Pfr can be lost either through a temperature dependent dark reversion of Pfr to Pr, or through an irreversible "destruction" of phytochrome

to an inactive form (Quail, 1976). Some evidence suggests that there are two pools of phytochrome present in germinating seeds, a small active or seed-phytochrome pool that does not undergo "destruction", and a bulk inactive or seedling-phytochrome pool that does (Kendrick et al., 1969). Pr is normally thought to be the stable form of phytochrome, however, in some seeds Pr produced by far red irradiation is capable of a rapid conversion back to Pfr in darkness (Boisard et al., 1968; Kendrick et al., 1969). This inverse dark reversion is thought to explain the need for repeated exposures of far red light to prevent the germination of dark germinating seeds such as lettuce cv. May Queen.

Phytochrome has been linked to two distinct types of photoresponses. These are the induction-reversion response and the high irradiance response (HIR) (Quail, 1976).

All positively photoblastic seeds exhibit the induction-reversion response (Taylorson and Hendricks, 1977). That is, the germination response can be saturated by a brief pulse of low intensity red, and can be reversed by a short exposure to far red light. Below saturation, the law of reciprocity holds i.e. the magnitude of the germination response is equal to the intensity multiplied by the duration of the light dose. Thus the induction-reversion response is energy dependent. Once the photostationary state has been established, the germination response becomes independent of irradiance. However, some species are known to require repeated exposures to red light to permit full germination (Toole, 1973). In these cases repeated red irradiations are thought to maintain active Pfr against a rapid dark reversion of Pfr to Pr (Taylorson and Hendricks, 1977).

In contrast to the induction-reversion response, the HIR system exhibits irradiance dependence even after a photostationary state has been established (Quail, 1976). In addition, the photoresponse is related to the time period over which the irradiation occurs (Smith, 1975). The HIR is thought to be responsible for the inhibition of germination in negatively photoblastic seeds by repeated or long exposures to white or far red light (Taylorson and Hendricks, 1977). The HIR has also been shown to be operating in both positively photoblastic (e.g. lettuce cv. Grand Rapids) and non-photoblastic (e.g. lettuce c.v. May Queen) seeds and can override the promotive effects of the induction-reversion response (Boisard et al., 1968; Negbi et al., 1968). The blue and far red wavelengths have been found to be most inhibitory, however, blue is much less effective than far red (Hartmann, 1966). Phytochrome is believed to be involved in the response to far red light but another photoreceptor may be involved in the response to blue (Smith, 1975).

The photosensitivity of most positively photoblastic seeds depends on the length of imbibition given before exposure to a specific light treatment (Smith, 1975). The general pattern observed is a sharp rise in photosensitivity as imbibition begins, followed by a peak and an eventual decline in photosensitivity. If dark imbibition is extremely prolonged, photosensitivity may be completely lost. The relationship between the length of imbibition and photosensitivity varies greatly between species, varieties and different lots of the same variety (see Smith, 1975). It is thought that the eventual decrease in photosensitivity is related to the induction of a secondary dormancy (Karssen, 1967).

As mentioned previously, Pfr is the active form of phytochrome. The interaction of Pfr with some unknown component (X) ultimately brings about germination (Smith, 1975). Pfr must be present in the seed for a certain length of time to promote germination. Thus, if far red light follows immediately after red irradiation, the promotive effect of red light is lost. However, if time is allowed to elapse between the red and far red treatments, far red light eventually becomes unable to reverse the effects of red irradiation. The time required for this escape from far red reversibility differs among species and varieties. In lettuce cv. Grand Rapids, a 50% loss of photoreversibility occurs in about 9 hours (Borthwick et al., 1954). A much more rapid effect of phytochrome has been demonstrated using subthreshold levels of gibberellic acid and red light to stimulate the germination of cv. Grand Rapids (Bewley et al., 1967). Under these conditions, escape from far red reversibility was found to occur within five minutes.

The mechanism of Pfr action is not yet understood. One hypothesis is that phytochrome acts through the activation or repression of specific genes (Mohr, 1966). However, rapid phytochrome effects such as the one mentioned previously as well as others (Haupt, 1972) suggest that gene activation cannot be the primary site of Pfr action. A more widely accepted hypothesis is that Pfr acts on membrane permeability. It has been proposed that phytochrome action is akin to a specific permease for an important metabolite and that this action is driven directly by the photoconversions of phytochrome (Smith, 1970).

The effect of Pfr on an individual seed is thought to be a threshold (all or none) response (Mohr, 1972). It is generally assumed that germination is determined by the number of Pfr molecules present

in the seed (Toole, 1973). Thus the germination response will vary with the total phytochrome pool and the degree of photoconversion. Different seeds may also vary in their response to a given number of Pfr molecules (Toole, 1973). It has also been suggested that an increase in temperature raises the minimum Pfr level required for germination (Spruit and Mancinelli, 1969).

The existence of Pfr in dark germinating seeds (e.g. lettuce cv. May Queen) has been clearly established (Boissard et al., 1968). It is commonly assumed that dark germinating seeds contain high levels of Pfr compared to light requiring seeds (Smith, 1975).

Dark germination levels have been shown to be influenced by the illumination conditions during maturation on the mother plant (McCullough and Shropshire, 1970). Seeds of Arabidopsis thaliana matured under lights rich in red showed higher dark germination levels than seeds matured under lights rich in far red. In addition, dark germination levels of many species including lettuce can be increased by a period of dry storage. It has been found that dry storage in the light increases dark germination levels of lettuce cv. Grand Rapids more rapidly than dry storage in the dark (Evanari and Neumann, 1953).

1.3 Temperature and Seed Germination

For all quiescent seeds, there exists a range of temperatures over which germination can occur. This range of temperatures is usually described in terms of the cardinal temperatures, that is the minimum, maximum and optimal temperatures for germination. The optimal temperature is usually defined as the temperature at which the highest percentage germination is obtained in the shortest time (Mayer and Poljakoff-Mayber, 1975).

Germination has been reported to occur at temperatures as high as 48C (Knapp, 1967), however, in most species the maximum is considerably lower than this. In lettuce the maximum temperature for germination typically falls in the range of 30-35C. The minimum temperature for germination is thought to be well above 0C for most temperate species (Heydecker, 1977); however, germination at 0C has been reported in species such as lettuce (Nichols and Heydecker, 1968). Because germination occurs very slowly at low temperatures, the minimum temperature for germination is usually poorly defined.

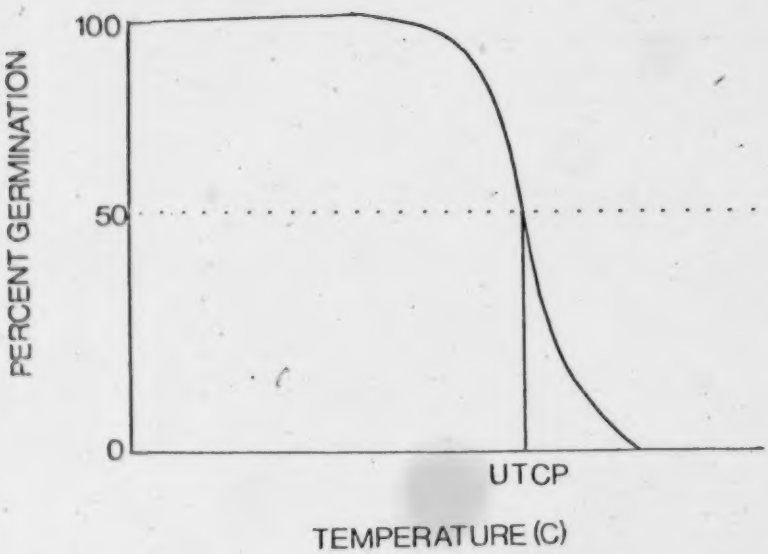
The relationship of the rate of germination to temperature is linear (Hegarty, 1973) or nearly so (Thompson and Fox, 1976) over a wide range of temperatures. However, the relation of the rate of germination to temperature is not a Q_{10} one. The increase in germination rate with temperature means that the optimal temperature for germination falls at the highest temperature that permits full germination.

Using thermogradient bars that allow germination tests to be made along a temperature gradient of ca 5-40C, Reynolds and coworkers have amassed considerable data on the relationship between temperature and germination (Thompson, 1970; Reynolds and Thompson, 1971; 1973; Reynolds, 1973). In most species the maximum temperature for germination was found to occur at a relatively low temperature (ca 30C), often lower than the thermal death point for that species (Thompson, 1970). As temperatures rose above the optimum, germination was found to decline very abruptly. In lettuce, germination fell from near 100% to almost zero with an increase of only 1 or 2C (Reynolds and Thompson, 1971).

The precise position of this decline on a temperature scale can be defined by determining the temperature at which germination has been reduced to some arbitrary level, typically 50% (Thompson, 1970). This parameter, known as the upper temperature cut-off point (UTCP) (Reynolds and Thompson, 1971), ceiling temperature (Heydecker and Joshua, 1977), or upper temperature limit (Biddington and Thomas, 1978), has been shown to be reproducible for a given lot of seeds. However, the UTCP varies among species (Thompson, 1970), varieties (Reynolds, 1973), and even among different lots of the same variety (Smith, 1975). Figure 1 shows the relationship of the UTCP to percent germination and germination temperature.

As mentioned previously the maximum temperature or UTCP for the germination of lettuce is known to occur in the range of 30-35C. However, the germination of lettuce is controlled by a complex interaction of light and temperature. In the light sensitive cultivar Grand Rapids, germination at high temperatures (ca 30-35C) is prevented in both the light and the dark. This inhibitory effect of high temperatures was initially termed "heat dormancy" (Evanari, 1952), however the terms thermodormancy (Gutterman et al., 1972; Negm et al., 1972), and thermoinhibition (Vidaver and Hsiao, 1975) have also been applied. At lower temperatures (ca 20-30C) the seeds germinate in the light but germinate poorly or not at all in the dark. At these temperatures the seeds may be termed photodormant. At progressively lower temperatures dark germination levels rise, so that full germination can often be obtained even in a light sensitive cultivar such as Grand Rapids. The precise relationship between dark germination levels and temperature varies greatly among different lots of cv. Grand Rapids (see Smith, 1975).

Fig. 1 Relationship of the UTCP to percent germination and temperature.


100
50
0
PERCENT GERMINATION
UTCP
TEMPERATURE (C)

Lettuce cultivars such as Great Lakes have been called light insensitive because they exhibit full germination in the dark at ca 20-25C (Toole, 1973). Nevertheless, at higher temperatures (ca 25-35C) red light does promote germination above the level of dark controls (Takeba and Matsubara, 1976). Therefore, it has been suggested that the difference between light sensitive Grand Rapids and other so-called light insensitive cultivars is quantitative, depending on temperature, rather than qualitative (Evanari, 1961).

Reynolds has shown that the complex germination behavior of lettuce can be described in terms of changes in the UTCP. Short red irradiations raise the UTCP in both light sensitive (Reynolds, 1973) and so-called light insensitive cultivars (Reynolds and Thompson, 1973).

The promotive or inhibitory effects of other chemical or physical factors can also be related to shifts in the UTCP (Reynolds and Thompson, 1971; 1973; Reynolds, 1973; 1975a and b; 1977). It has been postulated that the ultimate effect of germination promoters is to raise the UTCP, while inhibitors of germination decrease the UTCP (Reynolds, 1973). This mechanism has been demonstrated for the germination modifying effects of abscisic acid (ABA), kinetin (KIN) and gibberellic acid (GA_3) (Reynolds and Thompson, 1971; 1973). Increasing concentrations of these growth regulators caused progressively larger shifts in the UTCP. Similarly, the inhibition of germination by solutions of high osmotic pressure was also related to a decline in the UTCP (Reynolds, 1975a).

The suppression of germination in lettuce was found to occur in two distinct ways. Extremes of pH inhibited germination at all temperatures but did not affect the position of the UTCP (Reynolds,

1975b). This was described as a "toxic" effect of pH extremes. In contrast, the inhibitory effect of low concentrations of ABA was caused by a decline in the UTCP that permitted full germination to occur at lower temperatures (Reynolds and Thompson, 1973). This effect was termed true "inhibition". The suppression of germination by ABA involved both effects. ABA was "inhibitory" at low concentrations but became "toxic" at high concentrations.

1.4 Interactions of Light, Temperature and Applied Growth Regulators

Numerous workers have studied the effects of applied growth regulators on the germination of lettuce. The following discussion is not meant to be a survey of this vast literature but simply a summary of the hormonal effects that pertain directly to this thesis. A more complete survey of this area can be found in several recent reviews (Jones and Stoddart, 1977; Ketring, 1977; Thomas, 1977; Walton, 1977).

It has previously been noted that short exposures to red light raise the UTCP in lettuce. For descriptive purposes it is convenient to describe lettuce as having two upper temperature cut-off points, one for germination in the dark ($UTCP_D$) and one for germination in the light ($UTCP_L$).

In the absence of light the germination of photodormant lettuce seeds can be stimulated by application of GA_3 or other gibberellins (Kahn et al., 1957; Ikuma and Thimann, 1963). The effectiveness of different gibberellins varies greatly. A mixture of GA_4 and GA_7 is reported to be 100 times more active than GA_3 (Ikuma and Thimann, 1963). Subthreshold levels of GA_3 and light are reported to be highly synergistic rather than additive in effect (Bewley et al., 1967; 1968).

GA_3 plus saturating doses of red light has little or no effect on the germination of thermoinhibited seeds (Dunlap and Morgan, 1977; Keys et al., 1975; Reynolds, 1973). Thus GA_3 is able to increase the $UTCP_D$ but has little effect on the $UTCP_L$. It has also been found that GA_3 or GA_{4+7} can promote dark germination up to but not significantly beyond the $UTCP_L$ (Reynolds, 1973).

Cytokinins such as kinetin are also able to promote dark germination in photodormant lettuce, but the promotion is much less than that obtained with GA_3 (Leff, 1964; Reynolds, 1973). The effect of kinetin on dark germination is strongly synergistic with traces of light (Miller, 1958; Reynolds, 1973) or GA_3 (Ikuma and Thimann, 1963; Reynolds, 1973). Unlike GA_3, kinetin is able to promote the germination of seeds held at thermoinhibitory temperatures (Haber and Tolbert, 1959; Smith, 1968; Reynolds, 1973). This effect occurs in the dark and is synergistic with light or GA_3 (Haber and Tolbert, 1959; Keys et al., 1975). Reynolds (1973) found that a combination of red light plus kinetin raised the $UTCP_L$ to the thermal death point for lettuce (ca 42C) (Reynolds, 1973). The most dramatic effect of cytokinins is their ability to reverse the effects of inhibitors such as abscisic acid (Aspinall et al., 1967; Khan, 1975). Abscisic acid reduces germination in both the light and the dark (Reynolds, 1973). Kinetin is able to reverse both effects (Reynolds, 1973). In contrast, GA_3 is ineffective or only slightly effective in overcoming the effect of ABA (Khan and Waters, 1969; Reynolds, 1973).

Reynolds (1973) has demonstrated that low concentrations of ABA, KIN or GA_3 may have no effect on germination or considerable effect, depending entirely on the temperature at which the test was done and

the illumination conditions used. With rare exceptions most previous germination tests have been conducted at only one or a few arbitrarily chosen temperatures. Reynolds (Reynolds and Thompson, 1971; 1973; Reynolds, 1973) has suggested that this has led to inconsistencies in the literature regarding the reported effectiveness of various growth regulators.

1.5 The Induction of Photodormancy and Secondary Dormancy in Lettuce

As temperatures rise above the UTCP, germination declines, that is the seeds become thermoinhibited (thermodormant). However, if thermoinhibited seeds are returned to lower temperatures, full germination can usually be restored (Carpita and Nabors, 1976; Dunlap and Morgan, 1977; Keys et al., 1975; Vidaver and Hsiao, 1975). Therefore, in the nomenclature of Harper (1957), the dormancy is "enforced" by high temperatures. Because of this, it has been suggested that the term thermoinhibition is more appropriate than thermodormancy for describing the enforced effects of high temperatures (Vidaver and Hsiao, 1975).

If seeds are imbibed for prolonged periods (e.g. 1-10 days) at thermoinhibitory temperatures, they may eventually lose their capacity to germinate when returned to lower temperatures. This "induced" dormancy (Harper, 1957) may reduce the levels of both dark germination (Borthwick et al., 1952; 1954) and germination in the light (Burdett, 1972a and b; Gutterman et al., 1972).

Many workers (see Toole, 1961) have shown that dark germination at ca 20C in cv. Great Lakes can be reduced by several days incubation at ca 35C. After such treatments the germination behavior of this so-called light insensitive cultivar resembles that of light

sensitive Grand Rapids. That is, the seeds are photodormant at moderate temperatures (ca 20C). Similarly dark germination levels in cv. Grand Rapids can be suppressed by incubations at high temperature (Borthwick et al., 1952; 1954). In addition, when seeds of cv. Grand Rapids are exposed to red light and incubated at ca 35C, the promotive effect of the light treatment on germination at 20C is progressively lost as the time spent at 35C increases (Borthwick et al., 1954). Thus high temperature incubations are able to induce a photodormancy in both light sensitive and so-called light insensitive cultivars.

Since this dormancy can be overcome by exposure to light, the induction of photodormancy has commonly been attributed to the thermal reversion of Pfr to Pr (Gutterman et al., 1972; Scheibe and Lang, 1965). More recently Heydecker and Joshua (1976; 1977) demonstrated that the induction of photodormancy in the cultivars Great Lakes and Cobham Green is related to a decline in the UTCP. These cultivars showed full germination in light or darkness at 25C, but only a low level of germination at 30C. When seeds were incubated at 30C for seven days, subsequent dark germination at 25C was reduced. However, if the seeds which did not germinate in the dark at 25C were transferred to 15C, full germination occurred. Thus the induction of photodormancy appeared to be related to a decline in the $UTCP_D$. The effect was greater in seeds incubated in the dark than in seeds incubated in the light. In these cultivars incubation at 30C has no effect on subsequent germination in the light at 25C.

The imposition of a light requirement on non-photodormant seeds by imbibing them in solutions of high osmotic strength is well known (Borthwick, 1965; White et al., 1972). If seeds made photodormant

in this way are transferred back to water, the light requirement is abolished. Therefore this effect may be considered to be an enforced dormancy. However, in one report (Kahn, 1960) a photodormancy was induced in seeds of cv. Grand Rapids by holding them in osmotica at 20C for varying periods. When these seeds were subsequently transferred to water dark germination levels were reduced. A similar effect has recently been reported for the light insensitive cultivar Mesa 659 (Khan, 1978).

Several reports have shown that incubating seeds of cv. Grand Rapids at ca 30-35C reduces subsequent germination at lower temperatures (ca 20-26C) in both the light and the dark. The length of 30C incubation required to reduce germination in response to R (at 20C) to less than 50%, varied from 36 hours (Burdett, 1972a) to more than three days (Vidaver and Hsiao, 1975). Seeds that were unable to germinate in response to red light at 20C were considered to be secondarily dormant. Secondary dormancy was also induced in photodormant seeds incubated at 20C in the dark (Vidaver and Hsiao, 1974; Speer et al., 1974). Dormancy was not induced in seeds held under anaerobic conditions at 20C (Vidaver and Hsiao, 1975).

Secondarily dormant seeds are unable to germinate in response to R alone. Prolonged or repeated exposures to R have no effect on germination (Speer et al., 1974). However, combinations of R plus KIN (Speer and Tupper, 1975), GA_3, ethylene or thiourea (Speer et al., 1974) were found to restore at least partial germination to secondarily dormant seeds. GA_3 was unable to substitute for light in this effect (Speer and Tupper, 1975; Speer et al., 1974).

Vidaver and Hsiao (1975) found that exposing seeds to red light prior to 30C incubation had little effect on the induction of secondary dormancy. They concluded that the induction of secondary dormancy is independent of the phytochrome status of the seed and occurs whenever germination is suppressed for a sufficiently long period.

Vidaver and Hsiao (1974) also found that after two days of dark storage at 20C, GA_3 became unable to promote dark germination. However, red light remained effective in promoting germination for several days longer. Initially red light and GA_3 had been equally effective in promoting germination of untreated seeds at 20C. As the period of incubation at 20C increased, both red light and GA_3 became ineffective. However, red light plus GA_3 promoted full germination even in seeds incubated for 10 days. They concluded that secondary dormancy resulted from a blockage of a non light sensitive pathway (that was initially stimulated by GA_3) with a concomitant loss of endogenous gibberellin activity required for a light sensitive pathway.

Prolonged incubations under non germinating conditions have also been conducted on lettuce cultivars other than Grand Rapids. The induction of a secondary dormancy that prevents germination in response to R at ca 20C has not been reported in these cultivars (Gray, 1977; Dunlap and Morgan, 1977; Heydecker and Joshua, 1976; 1977; Smith et al., 1968; Takeba and Matsubara, 1976). However, prolonged high temperature incubations have been shown to reduce the rate of germination (i.e. the time required for first radicle emergence) in many of these cultivars, as well as in cv. Grand Rapids (Carpita and Nabors, 1976; Gray, 1977; Heydecker and Joshua, 1976; 1977).

1.6 Objectives

The importance of temperature in regulating the germination of lettuce has been well established. The induction of photodormancy, and the effects of germination modifying factors have been explained in terms of shifts in the UTCP. However, the relationship between temperature and the germination of secondarily dormant seeds is less well understood. In all previous investigations of secondary dormancy, germination was monitored at only one temperature. Therefore, in this investigation, experiments were conducted over a wide range of temperatures to determine the relationship between the $UTCP_L$ and the induction of secondary dormancy in lettuce cv. Grand Rapids. The effects of temperature and light on the induction of secondary dormancy were also examined.

Previous reports have indicated that secondary dormancy occurs in cv. Grand Rapids but not in other lettuce cultivars. Experiments were conducted to test this observation and to investigate other differences between the germination behavior of cv. Grand Rapids and cvs. Great Lakes or New York.

The implications of the results of these experiments are discussed in terms of dormancy mechanisms in lettuce.

2.0 Materials and Methods

2.1 Seeds

Lettuce seeds (Lactuca sativa L.) of the varieties Grand Rapids (I), New York, and Great Lakes were obtained from the Robertson Seed Co., Edmonton, Alberta. An additional lot of cv. Grand Rapids (II) was obtained from the Carolina Biological Supply Co., Gladstone, Oregon.

All seeds were examined before use, and damaged, abnormally small or off-colour seeds were removed. This practice allowed 100% germination levels to be routinely obtained, and also helped reduce fungal and bacterial infection during prolonged incubation of the seeds. After prolonged incubations (i.e. 4 days or more) fungal or bacterial infection was usually apparent on one or two isolated seeds per petri dish. Infected seeds were not included in germination counts.

To facilitate the handling of large numbers of seed samples, lots of approximately 100 seeds were measured out by volume and dispensed into individual vials. Throughout this thesis the term "samples" is used to refer to seed lots of approximately 100 seeds. All seeds were stored in the dark over a dessiccant at approximately 3C.

Chemicals

Aqueous solutions of gibberellic acid (100 ug/ml), kinetin (10 ug/ml) and polyethylene glycol 6000 (25% W/V) were prepared using glass distilled water. Gibberellic acid (90% GA_3 activity and kinetin were from the Sigma Chemical Co. Polyethylene glycol 6000 (PEG 6000) was from Fisher Scientific Co.

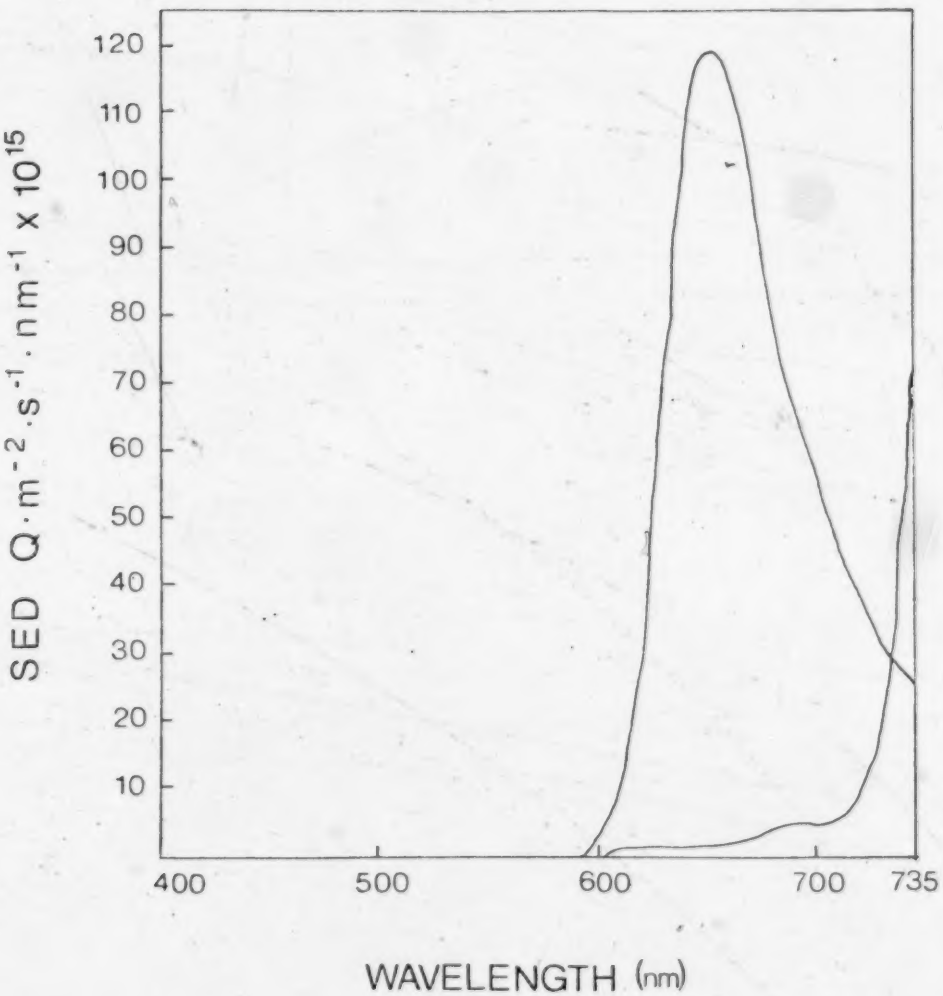
2.2 Light Sources

All manipulations of the seeds were conducted in a darkroom under a green photomorphogenically inactive safelight, consisting of two 15 watt cool white fluorescent tubes wrapped in a triple layer of No. 39 primary green Cinemoid (Rank Strand Electrics Ltd., London, England). Red irradiation (7.5×10^{18} $Q \cdot m^{-2} \cdot sec^{-1}$) was provided by filtering the light from four 15 watt cool white fluorescent tubes through a double layer of No. 14 ruby Cinemoid. Far red light (1.1×10^{18} $Q \cdot m^{-2} \cdot sec^{-1}$) was obtained by filtering the light of four 75 watt incandescent bulbs through 10 cm water and one layer each of No. 5 orange and No. 20 deep blue Cinemoid. The spectral energy distribution of the red and far red light sources (Fig. 2) was measured with a Quantaspectrometer model QSM-2500 (Techtum Instrument, Sweden). Unless otherwise specified, all light treatments were of five minutes duration. Imbibition and prolonged incubations of seeds were normally done in complete darkness. However, in certain experiments (which are so noted), seeds were repeatedly exposed to R (i.e. 5 min. R every 30 min.) or were exposed to continuous cool white fluorescent light.

2.3 Temperatures

High temperatures (32 or 35C) were provided by a walk-in chamber fitted with a dim green safelight. For high temperature incubations seed samples were dispensed into petri dishes that had been equilibrated at the chamber temperature. Seven smaller reach-in growth cabinets provided temperatures of 15, 20, 22, 24, 26, 28, and 30C.

Fig. 2 Spectral energy distribution (SED) of the red and far red light sources.


SED Q·m⁻²·s⁻¹·nm⁻¹ x 10¹⁵
120
110
100
90
80
70
60
50
40
30
20
10
400
500
600
700
735
WAVELENGTH (nm)

Temperatures were usually not uniform throughout the interior of each growth cabinet. To correct for this temperature variability, areas of known, stable temperature were determined within each growth cabinet, and seed samples were placed only within these areas. Localized temperature differences were determined as follows. Stoppered, 250 ml flasks containing 50 ml of water were distributed throughout the interior of each growth cabinet. The temperature of the water within these flasks was measured with a single mercury thermometer. This procedure also eliminated the variability in temperature measurements caused by the rapid heating-cooling cycle of the growth cabinets. Temperatures within the chosen restricted areas of each growth cabinet varied less than ±0.5C from the desired temperature. Temperatures within the petri dishes themselves were occasionally checked using a fine copper-constantan thermocouple connected to a chart recorder or a digital multimeter. After several hours equilibration at chamber temperatures, temperatures within the petri dishes varied less than ±0.25C. Light treatments and other manipulations of the seeds were usually conducted in a darkroom at approximately 22C. In seed transfer experiments, lengthy manipulations of the seeds were required. For seeds incubated at 32 or 35C these manipulations were done within the walk-in chamber, at the incubation temperature.

2.4 Germination Testing Procedures

2.4.1 General Procedures

Approximately 100 seeds per replicate were distributed in 9 cm petri dishes containing two Whatman No. 1 filter discs and 5 ml distilled water or test solution. Groups of 3 to 5 petri dishes were

wrapped in a single layer of aluminum foil and placed at specific temperatures. Light treatments were normally conducted after one hour imbibition. Unless otherwise specified, germination, as measured by radicle emergence, was determined after 5 days in seeds placed at 15C and after 3 days in seeds placed at all other temperatures. At 15C the rate of germination (as determined by the time to first radicle emergence) was slower than at higher temperatures. Therefore, additional germination time was provided for seeds placed at 15C. Normally germination at all temperatures was complete within 24 to 36 hours. However, certain treatments, such as prolonged incubation under non-germinating conditions slowed the rate of germination. Therefore, lengthy germination periods were used to ensure that germination was complete when counts were made. All data reported in this thesis are for the maximum germination obtained at a specific temperature.

2.4.2 Incubation Under Non-Germinating Conditions

In many experiments seeds were held fully imbibed for prolonged periods under conditions that did not permit germination. At the end of this "incubation" period, all petri dishes were opened and any seeds that had germinated were removed. The seeds then received some type of light or hormone treatment (e.g. R, FR, transfer to GA_3, KIN or fresh distilled water) and were placed at temperatures normally suitable for germination. In experiments involving prolonged exposure to light during the incubation period, the petri dishes were prevented from drying out by sealing them with parafilm.

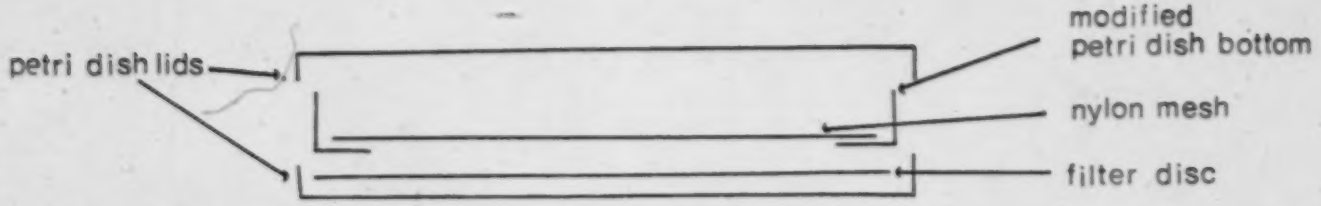
2.4.3 Transfer Experiments

Some experiments involved the transfer of seeds from one test solution to another. This was accomplished by two methods:

1) In experiments involving transfer from water to another solution (e.g. water → GA_3), the filter discs that the seeds rested on were lifted out, and placed to dry on a paper towel. After air drying for a few seconds the seeds were scraped with a spatula into fresh petri dishes.

2) In experiments involving the transfer of seeds from solutions of PEG 6000 to water, a thorough washing of the seeds was desired before transfer to the fresh solution. This was accomplished by means of transfer dishes (Fig. 3). Transfer dishes were prepared by cutting a hole in the bottom of a plastic petri dish, leaving only a 1 cm lip around the edge. The bottom of the petri dish was replaced with a fine nylon mesh and this assembly was placed in a petri dish lid containing one Whatman No. 1 filter disc and 5 ml test solution. The entire assembly was then covered with another petri dish lid. To move seeds from one solution to another, the transfer assembly was lifted out and the seeds were rinsed and dried three times before transfer to the fresh solution.

Fig. 3 Transfer dishes used for PEG 6000 to water transfers.


petri dish lids
modified
petri dish bottom
nylon mesh
filter disc

3.0 Results and Discussion

3.1 Germination response to the timing and duration of R treatment.

In a preliminary experiment, seeds of cv. Grand Rapids (II) were exposed to varying durations of R to determine the conditions needed to saturate the germination response (Fig. 4). A two minute R treatment was sufficient to induce full germination in seeds that had been imbibed 1.0 hour. In contrast, a five minute R irradiation was incapable of inducing 100% germination in seeds that had been imbibed for 0.5 hours. Therefore, to ensure saturation of the germination response, a combination of one hour imbibition and five minutes R irradiation was chosen as a standard experimental procedure. These conditions induced 100% germination at 24C in all 4 seed lots (i.e. Grand Rapids I, Grand Rapids II, New York and Great Lakes). If R irradiation was followed immediately by a 5 min FR treatment, the germination of cv. Grand Rapids (II) was reduced to less than 1%. Far red treatments were not conducted on the other seed lots.

3.2 The influence of light and temperature on the germination of several varieties of lettuce.

Seeds of three different lettuce cultivars were exposed to a variety of light and temperature conditions to compare their germination behaviors (Table 1). Germination in response to R was reduced to zero by incubation at 32C in all seed lots except cv. Grand Rapids (I). A temperature of 35C was required to completely suppress germination in this seed lot. It was apparent in all seed lots that as temperatures increased, dark germination levels declined more rapidly than

Fig. 4 Germination response to timing and duration of R treatment.

Seeds of cv. Grand Rapids (II) were imbibed at 24C for 0.5 (●) or 1.0 (■) hours before varying durations of R irradiation. Germination was determined after 48 hours at 24C. Each point represents the mean of 3 samples.

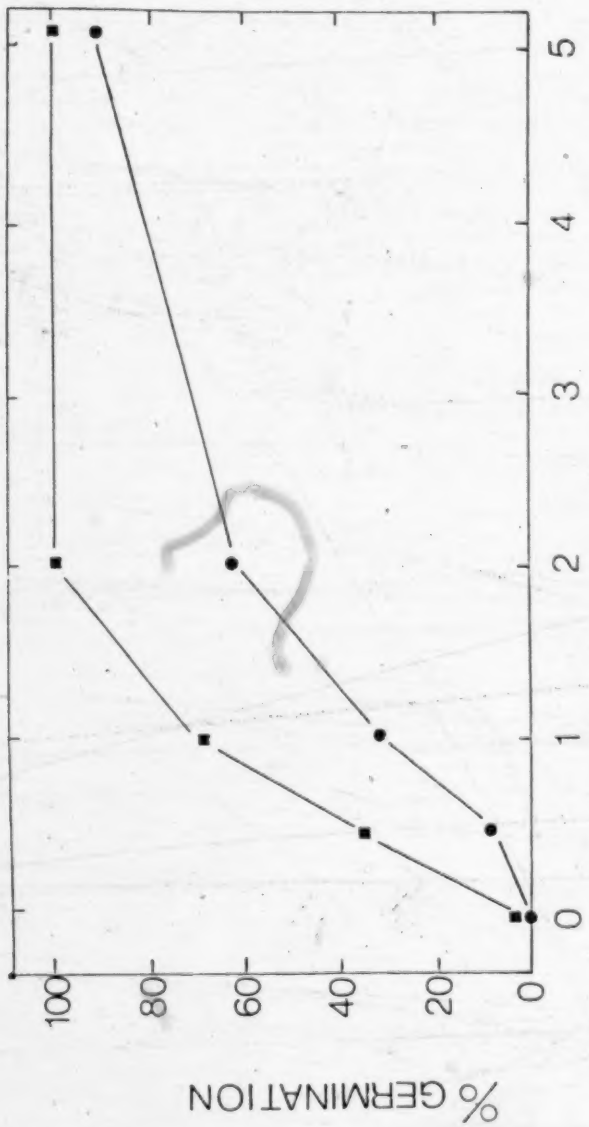

% GERMINATION
0
20
40
60
80
100
0
1
2
3
4
5
DURATION OF R IRRADIATION (min)

Table 1

The influence of light and temperature on the germination of several varieties of lettuce.

variety	light conditions	PERCENT GERMINATION Germination Temperature 20	26	30	32	35
Grand Rapids (I)	R	100	100	85	25	0
	D	100	68	17	0	0
Grand Rapids (II)	R	100	100	20	0	0
	D	23	0	0	0	0
Great Lakes	R	100	100	52	0	0
	D	100	100	1	0	0
New York	R	94	97	47	0	0
	D	60	38	0	0	0

Seeds were imbibed one hour at 22C before light treatment and transferred to the indicated temperatures. Germination was determined after 48 hours. Each value represents the mean of 2 samples.

germination in response to R. Thus at 30C, R promoted germination even in the so-called light insensitive cultivar Great Lakes. Similar results have been obtained by other workers (Takeba and Matsubara, 1976).

Dark germination levels differed considerably between the two lots of cv. Grand Rapids. Seeds of cv. Grand Rapids (I) showed a high level of dark germination at temperatures up to 30C. In contrast, seeds of cv. Grand Rapids (II) germinated poorly in the dark, even at 20C.

The germination behavior of all four seed lots can also be described in terms of their upper temperature cut-off points. The $UTCP_L$ fell between 26 and 32C in all seed lots. In cvs. New York, Great Lakes, and Grand Rapids (I) the $UTCP_D$ could also be roughly estimated. However, the situation is less clear in the case of cv. Grand Rapids (II). Further experiments showed that in this seed lot, dark germination did not rise to 50%; even at lower temperatures (ca 10-15C). Thus the 50% figure cannot always be applied to work with $UTCP_D$. Biddington and Thomas (1978) used the 10% level of germination to define the UTCP in celery.

3.3 Effects of high temperature incubation on the subsequent germination of dark or R treated seeds.

High temperature incubations have been shown to reduce dark germination levels (Carpita and Nabors, 1976) and induce a secondary dormancy (Burdett, 1972a; Vidaver and Hsiao, 1975; Speer and Tupper, 1975) in seeds of cv. Grand Rapids. However, in these reports germination was determined at only one temperature, usually 20C.

Therefore an experiment was conducted to determine the effects of high temperature incubations on the germination of cv. Grand Rapids (I) over a wide range of temperatures.

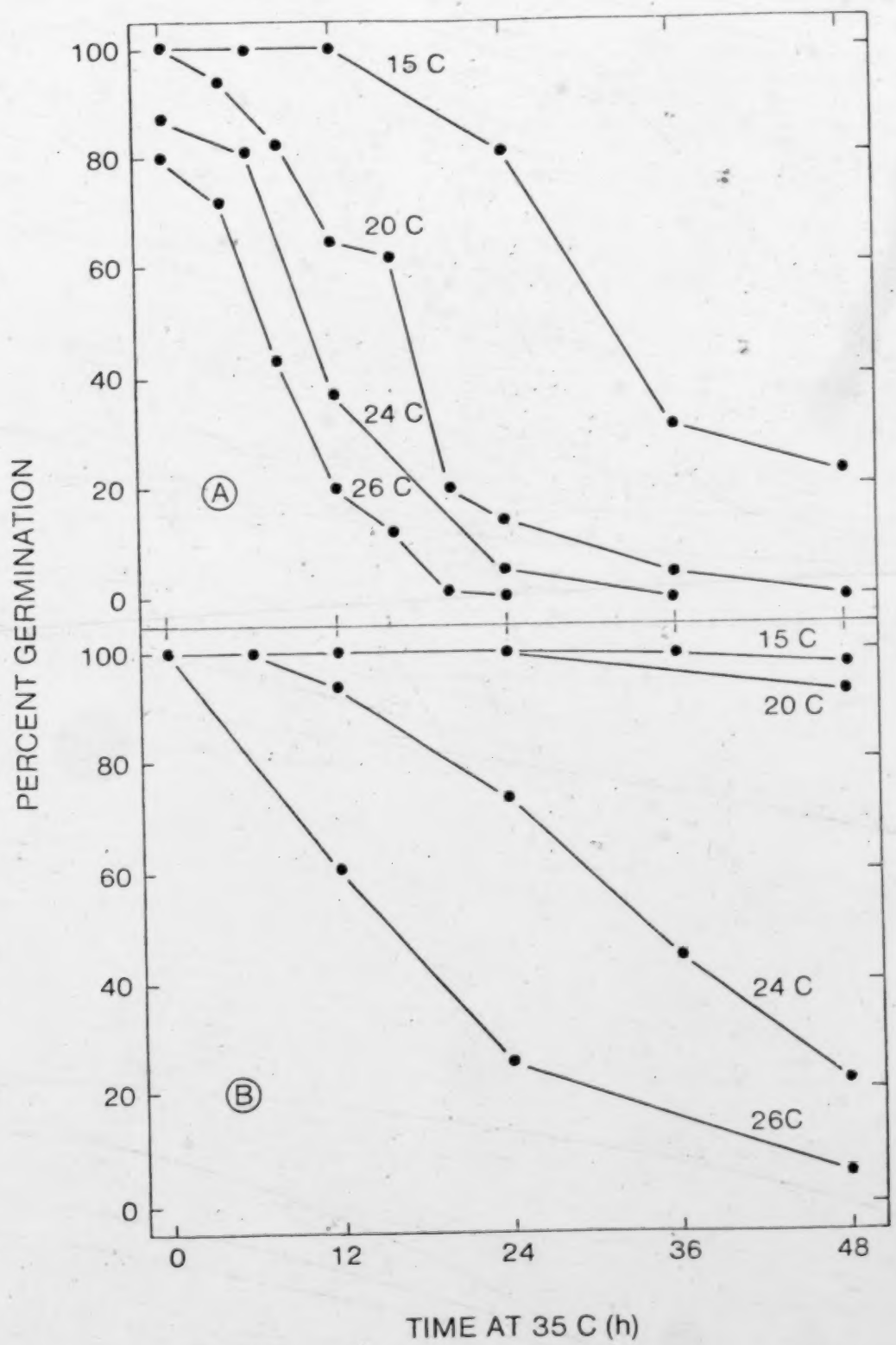
Short 35C incubations (e.g. 12 hours or less) reduced dark germination at 20, 24 and 26C but not at 15C (Fig. 5A). Longer incubations eventually reduced dark germination to a low level at all temperatures. Germination at 24 and 26C in response to R was also reduced by high temperature incubation. However, germination at 15 and 20C was almost unaffected (Fig. 5B).

These results indicate that in cv. Grand Rapids both the $UTCP_D$ and the $UTCP_L$ decline during incubation at thermoinhibitory temperatures. Heydecker and Joshua (1976; 1977) have previously shown that in cvs. Great Lakes and Cobham Green, the imposition of photodormancy was related to a decline in the $UTCP_D$. However, in their seeds germination in the light was not affected by high temperature incubation. A recent report (Biddington and Thomas, 1978) has shown that high temperature incubations reduce the germination of celery at 22C through an effect on the $UTCP_L$.

Some reports (Carpita and Nabors, 1976; Vidaver and Hsiao, 1975) have indicated that short high temperature incubations (e.g. less than 24 hours) have little or no effect on subsequent germination in the light. It is apparent from the results presented in Fig. 5B that brief high temperature treatments may have a considerable effect on germination, but that this effect may not be apparent at low temperatures. Thus, after high temperature incubations it is important to test germination over a wide range of temperatures.

Fig. 5 Effects of high temperature incubations on the subsequent germination of dark or R treated seeds.

Seeds of cv. Grand Rapids (I) were incubated in the dark at 35C for varying periods and transferred to the indicated temperatures. R treated seeds (Fig. 4B) were irradiated at the end of the 35C incubation. Each point represents the mean of 3 samples.


PERCENT GERMINATION
100
80
60
40
20
0
15 C
20 C
24 C
26 C
A
15 C
20 C
24 C
26C
B
0
12
24
36
48
TIME AT 35 C (h)

3.4 Effects of high temperature incubation on the $UTCP_L$ of cvs. Great Lakes, New York and Grand Rapids (I).

The results in Fig. 5B indicated that high temperature incubations reduced the $UTCP_L$ of cv. Grand Rapids (I). However, seeds incubated for 48 hours were not secondarily dormant, since full germination in the light could still be obtained at 15 or 20C. Therefore an experiment was done to determine the effect of longer incubations on the $UTCP_L$ of cvs. Great Lakes, New York and Grand Rapids.

High temperature incubations were conducted at the lowest temperature able to restrict germination to zero, under both light and dark germination conditions. This had previously been found to be 35C for cv. Grand Rapids (I) and 32C for all other seed lots (Table 1).

The $UTCP_L$s for untreated (0d) seeds of cvs. Great Lakes, New York and Grand Rapids (I) were found to be approximately 29, 30 and 31C respectively (Fig. 6). The germination of cv. Great Lakes was almost unaffected by high temperature incubations. In contrast, both cv. New York and cv. Grand Rapids showed considerable declines in their $UTCP_L$s, particularly within the first 12 hours of incubation. The $UTCP_L$ of cv. New York declined to approximately 25C after 2 days of incubation at high temperature, however longer incubations had little or no further effect on the $UTCP_L$. In cv. Grand Rapids, long incubations seemed to cause a flattening out of the germination curve (e.g. Fig. 6C, 4D), and eventually suppressed germination at all temperatures. Repeated exposures to R were unable to restore germination.

These results indicate that under the test conditions used, only cv. Grand Rapids became secondarily dormant. This confirms previous

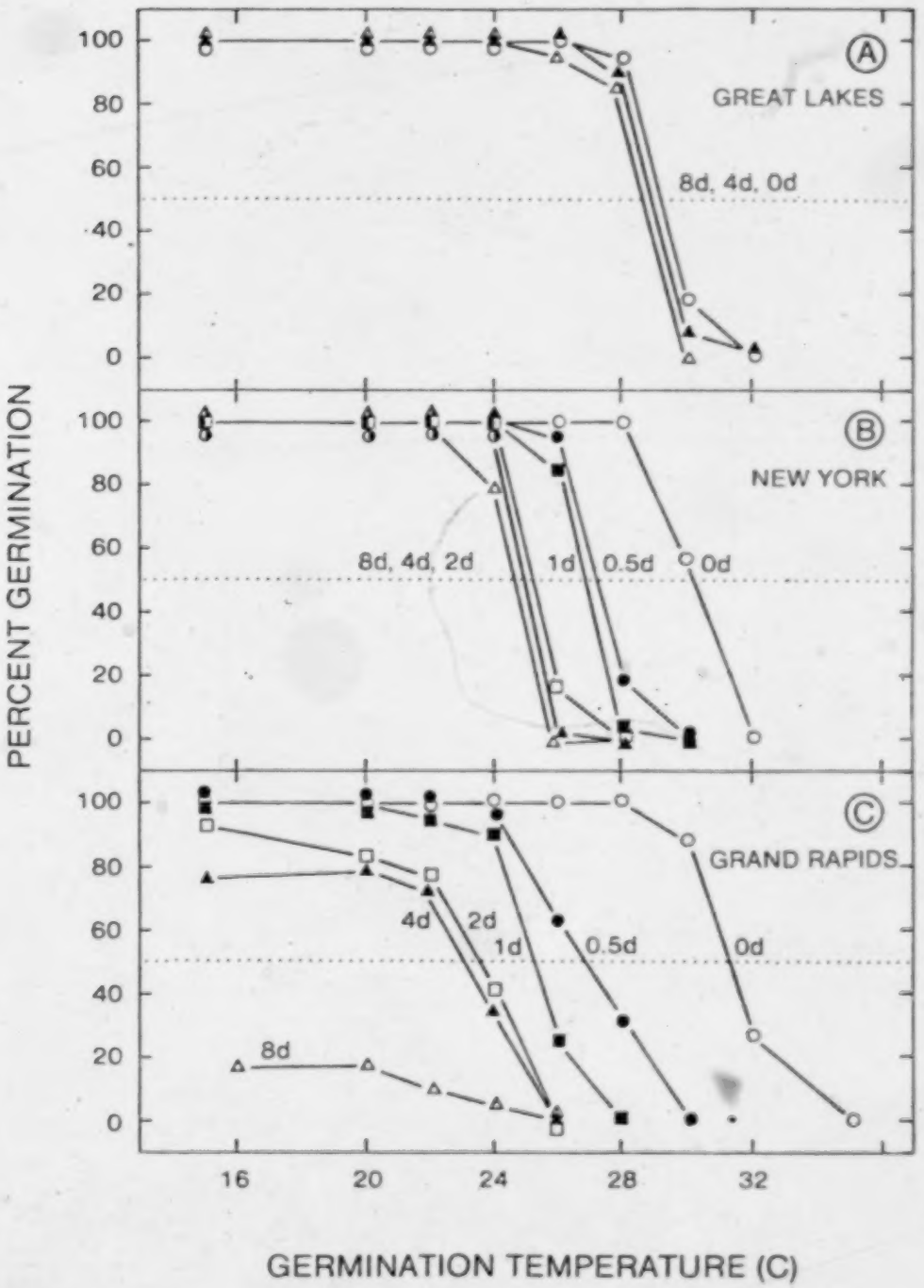
reports that indicated that secondary dormancy is not induced in cvs. New York or Great Lakes by high temperature incubation (Takeba and Matsubara, 1976; Smith et al., 1968; Heydecker and Joshua, 1976).

The induction of secondary dormancy in cv. Grand Rapids (I) appeared to occur in two stages. Initially germination was suppressed only in the vicinity of the $UTCP_L$. Longer incubations eventually suppressed germination at all temperatures. However, during this second stage of dormancy induction, germination seemed to decline at all temperatures simultaneously (e.g. Fig. 6C; 2, 4 or 8d), rather than just in the vicinity of the $UTCP_L$. Thus it is difficult to interpret the induction of secondary dormancy solely in terms of a decline in the $UTCP_L$. Instead, these two stages of dormancy induction resemble the "toxic" and "inhibitory" effects on germination described by Reynolds (1975b).

In addition to affecting absolute levels of germination, high temperature incubations are also known to affect the subsequent rate of germination (Gray, 1977; Carpita and Nabors, 1976; Heydecker and Joshua, 1977). In the present work the germination counts were timed so that differing germination rates had no effect on the final percentage germination obtained. However, in preliminary experiments to determine the time needed for maximum germination to occur, it was observed that prolonged (e.g. 8 d) high temperature incubations delayed the start of germination by up to 24 hours in all three cultivars. Thus, in cv. Great Lakes high temperature incubations had little or no effect on the percentage germination obtained (Fig. 6A), yet the rate of germination was decreased.

Fig. 6 Effects of high temperature incubation on the $UTCP_L$ of lettuce cvs. Great Lakes, New York and Grand Rapids (I).

Seeds were incubated at 32C (Great Lakes, New York) or 35C (Grand Rapids I) for periods of 0 to 8 days, exposed to R, and transferred to the indicated temperatures. Untreated seeds (0d) were imbibed one hour at 24C before R irradiation and transferred to germination temperatures. Each point represents the mean of 4 samples.


PERCENT GERMINATION
100
80
60
40
20
0
A
GREAT LAKES
8d, 4d, 0d
B
NEW YORK
8d, 4d, 2d
1d
0.5d
0d
C
GRAND RAPIDS
8d
4d
2d
1d
0.5d
0d
16
20
24
28
32
GERMINATION TEMPERATURE (C)

3.5 Effect of 32C incubation on dark germination of cv. Great Lakes.

The previous experiment showed that prolonged incubations at 32C had no effect on the germination of cv. Great Lakes in the light. However, it has been shown that high temperature incubations reduce the dark germination of this cultivar at 20C (e.g. Toole, 1959; 1961).

To determine the effects of high temperature incubation on dark germination in the present lot of cv. Great Lakes, seeds were incubated at 32C for 4 days and then transferred to 26C. Germination counts were made 48 or 120 hours later.

Fresh seeds germinated 100% at 26C within 48 hours. Similarly, seeds exposed to R at the end of the high temperature incubation germinated 100% at 26C within 48 hours. In contrast, zero germination had occurred in heat treated seeds after 48 hours at 26C. However, after 120 hours at 26C, 100% germination was obtained even in heat treated seeds. Therefore, 4 days of high temperature incubation had delayed the start of dark germination, but did not affect the percentage germination obtained at 26C.

If germination counts had been made only after 48 hours, it would have been reasonable to conclude that four days of high temperature incubation had induced a photodormancy in these seeds. Thus, it is conceivable that other reports on the induction of photodormancy in cv. Great Lakes, have failed to take into consideration the effects of high temperature incubation on the rate of dark germination.

3.6 Effects of dark incubation at 32, 24 and 15C on the $UTCP_L$ of cv. Grand Rapids (II).

Secondary dormancy can also be induced in seeds of cv. Grand

Rapids by prolonged dark incubations at 20C (Speer et al., 1974; Vidaver and Hsiao, 1974). Vidaver (1977) compared the rate of dormancy induction in seeds incubated at 20C (Speer et al., 1974) and at 30C (Vidaver and Hsiao, 1975) and concluded that secondary dormancy was induced more rapidly in seeds incubated at 30C. However, a meaningful comparison of these data is difficult because different experimental conditions were used in the two reports.

To obtain such a comparison, seeds of cv. Grand Rapids (II) were incubated at 32, 24, and 15C to compare the rate of dormancy induction at high and low temperatures (Fig. 7). Dark germination at these temperatures was less than 5% in this seed lot.

Four days of incubation at 32C totally suppressed the germination of cv. Grand Rapids (II) at all temperatures. In comparison, 8 days at 35C were required to produce the same effect in cv. Grand Rapids (I) (Fig. 6C).

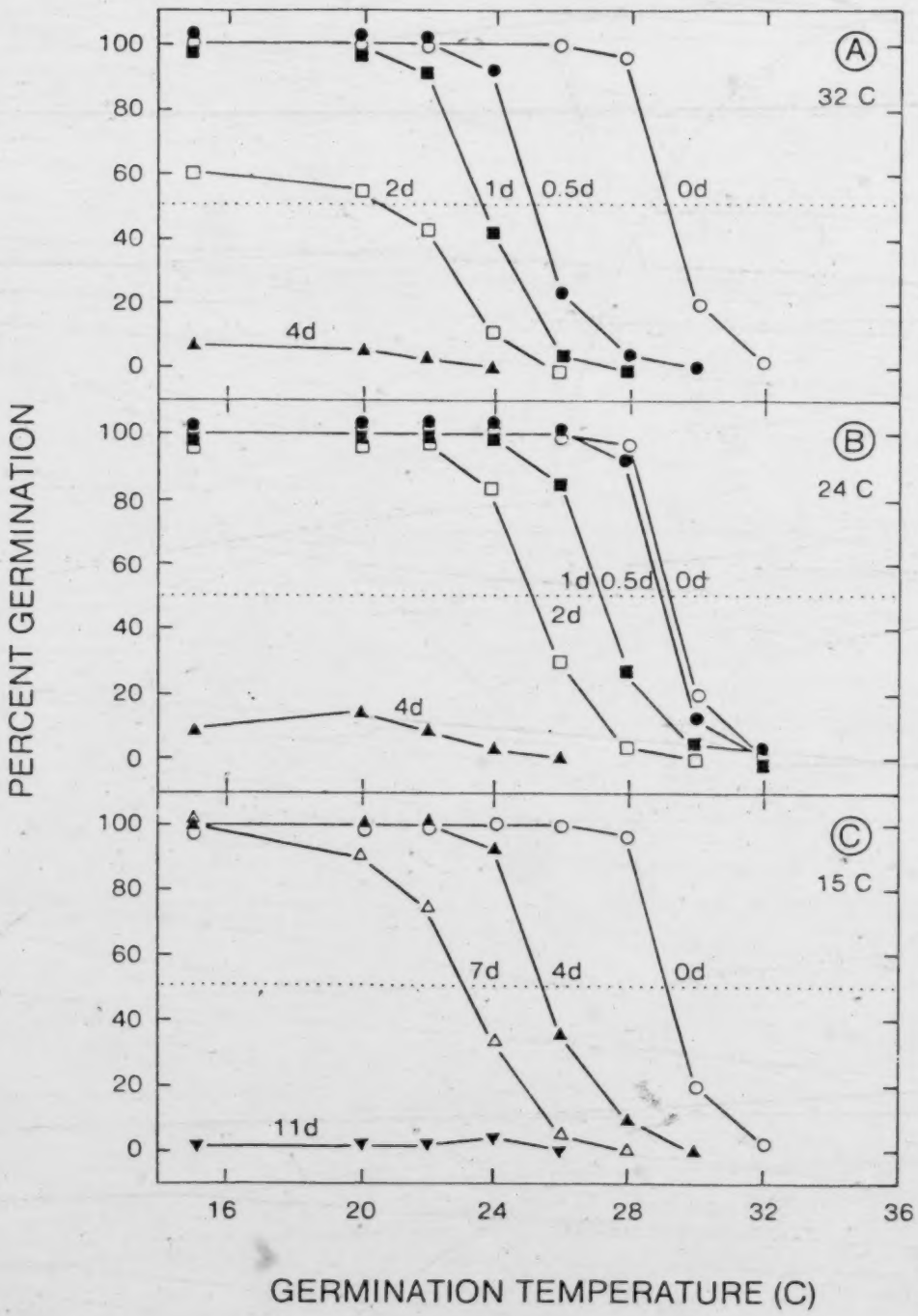
Secondary dormancy was also induced in seeds incubated at 15 or 24C. The rate of dormancy induction was clearly related to the temperature of the dark incubation. Twelve hours at 32C resulted in an $UTCP_L$ of approximately 25C. Two days at 24C or 4 days at 15C were required to produce the same effect.

The pattern of dormancy induction was also similar at all three incubation temperatures. Initially germination was suppressed only in the vicinity of the $UTCP_L$, however longer incubations eventually suppressed germination at all temperatures.

These results support the suggestion (Vidaver and Hsiao, 1975) that secondary dormancy occurs in lettuce whenever seeds are held under non-germinating conditions for a sufficient length of time.

Fig. 7 Effects of dark incubation at 32, 24 and 15C on the $UTCP_L$ of cv. Grand Rapids (II).

The procedure used was the same as in Fig. 6, except dark incubations were at 32, 24 and 15C. For untreated seeds (0d) each point represents the mean of 10 samples. All other points represent the mean of 4 samples.


A
32 C
2d
1d
0.5d
0d
4d
B
24 C
1d
0.5d
0d
2d
4d
C
15 C
7d
4d
0d
11d
100
80
60
40
20
0
16
20
24
28
32
36
PERCENT GERMINATION
GERMINATION TEMPERATURE (C)

3.7 Effect of incubation temperature on the rate of $UTCP_L$ decline.

The previous experiment indicated that the rate of dormancy induction was related to the temperature of the dark incubation. However, as germination temperatures decrease, the rate of germination also declines. In cv. Grand Rapids (II) the time to first radicle emergence was found to be approximately 13 hours at 24C, but greater than 36 hours at 15C. Therefore, it could be argued that dormancy induction is slower at low temperatures because the germination process takes longer to reach the stage at which dormancy can be imposed.

To investigate the temperature dependence of the dormancy inducing step itself, seeds were first incubated at 32C for 12 hours. This eliminated the effect of unequal germination rates, and brought the seeds to an early stage of dormancy induction (e.g. see Fig. 7A, 0.5d). Following this, the seeds received an additional 36 hours of incubation at 32, 24 or 15C.

The results shown in Fig. 8 indicate that the dormancy inducing step is temperature dependent. Compared to the control samples, incubation at 32C resulted in a considerable decline in germination levels. Incubation for 36 hours at 15C had little or no effect on the $UTCP_L$, while incubation at 24C was intermediate in effect.

3.8 Dark germination of cv. Grand Rapids (II) in response to GA_3.

Gibberellic acid can substitute for R in promoting the germination of photodormant lettuce seeds (Kahn et al., 1957; Ikuma and Thimann, 1963). An experiment was conducted to determine the effectiveness of GA_3 in promoting the dark germination of cv. Grand Rapids (II).

Fig. 8 Effect of incubation temperature on the rate of $UTCP_L$ decline.

Seeds of cv. Grand Rapids (II) were incubated at 32C for 12 hours, followed by an additional 36 hours at 32C (△), 24C (▲) or 15C (○). The control treatment (●) received 12 hours at 32C only. At the end of the incubation period all treatments were exposed to R and transferred to germination temperatures. Each point represents the mean of 4 samples.

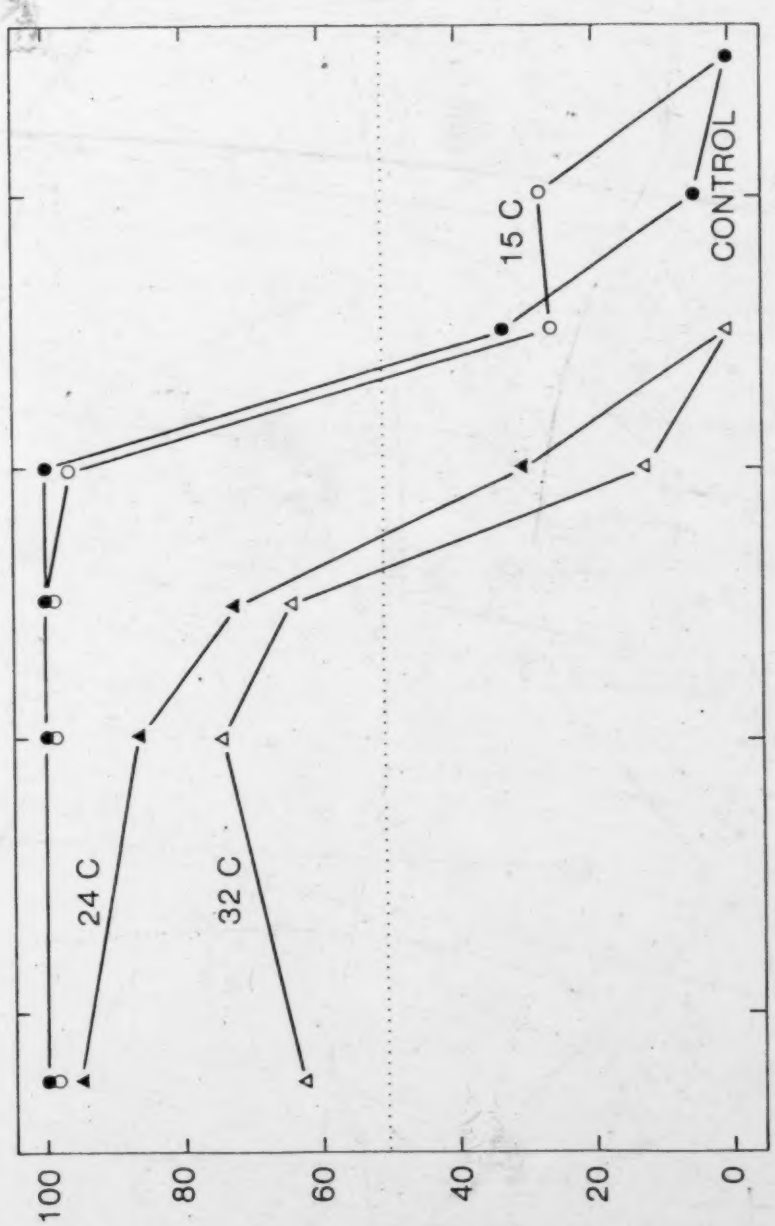
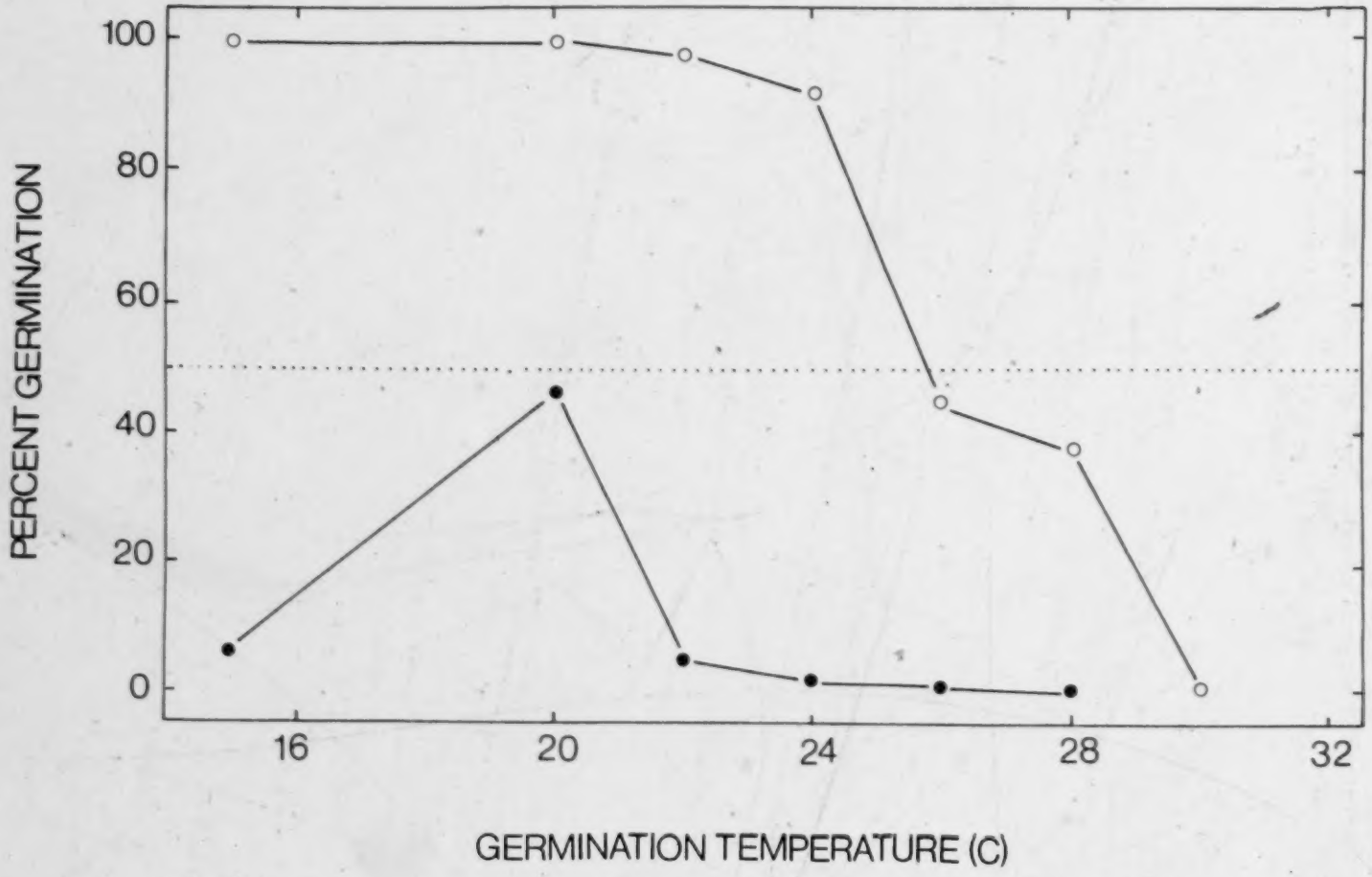

PERCENT GERMINATION
100
80
60
40
20
0
16
20
24
28
GERMINATION TEMPERATURE (C)
24 C
32 C
15 C
CONTROL

Fig. 9 Dark germination of cv. Grand Rapids (II) in response to GA_3.

Seeds were imbibed in the dark at the indicated temperatures in distilled water (●) or 100 ug/ml GA_3 (O). Each point represents the mean of 3 samples.


PERCENT GERMINATION
100
80
60
40
20
0
16
20
24
28
32
GERMINATION TEMPERATURE (C)

Compared to the water controls, 100 ug/ml GA_3 promoted dark germination at all temperatures up to and including 28C (Fig. 9). The resulting UTCP was approximately 26C. In comparison, the UTCP of seeds exposed to R was approximately 29C (Fig. 7, 0d). Therefore, this level of GA_3 was slightly less effective than R in promoting germination.

The water controls exhibited a low level of dark germination at all temperatures except 20C. Similar results were obtained when the experiment was repeated. In most lettuce seed lots, dark germination levels progressively increase as temperatures decrease (e.g. see Smith, 1975).

3.9 Germination of secondarily dormant seeds in response to KIN or GA_3.

Several reports (Speer et al., 1974; Speer and Tupper, 1975; Vidaver and Hsiao, 1975) have shown that both R+GA_3 and R+KIN are able to promote the germination of secondarily dormant seeds. However, in all previous experiments, germination was monitored only at 20C. Therefore, the following experiments were conducted to investigate the effects of GA_3 and KIN over a wider range of temperatures.

The results shown in Fig. 10 indicate that R+KIN was highly effective in promoting germination at temperatures up to and including 22C. R+GA_3 promoted germination somewhat at 15 and 20C but was much less effective than R+KIN. While both R+GA_3 and R+KIN were somewhat effective in promoting germination, neither treatment could restore germination to the levels obtained with fresh seeds (e.g. Fig. 7, 0d). Similar results were obtained with seeds of cv. Grand Rapids (I).

The transfer of secondarily dormant seeds to fresh dishes of distilled water also had a slight promotive effect on germination, compared to seeds left in their original petri dishes (e.g. compare Fig. 10, H_2O and Fig. 7A, 4d). This "transfer" effect will be described in greater detail in a later section.

Speer and Tupper (1975) found that R+KIN was about twice as effective as R+GA_3 in promoting the germination of secondarily dormant seeds at 20C. Two reports (Vidaver and Hsiao, 1974; Speer et al., 1974) showed that R+GA_3 was highly effective in promoting the germination of seeds made dormant by incubation at 20C. However, R+GA_3 was less effective in restoring germination to seeds made dormant by incubation at 30C (Vidaver and Hsiao, 1975).

In another experiment secondarily dormant seeds were transferred to H_2O, GA_3 or KIN but were not exposed to R. In this case zero germination occurred at all temperatures. Thus, in the absence of an R irradiation, GA_3 and KIN were ineffective in promoting the germination of secondarily dormant seeds. Similar results have been previously obtained (Speer et al., 1974; Speer and Tupper, 1975; Vidaver and Hsiao, 1974; Vidaver and Hsiao, 1975).

These results suggest that Pfr is present in secondarily dormant seeds exposed to R. However, Pfr seems incapable of promoting germination unless another factor such as KIN is supplied.

3.10 Reversal of R promoted germination by incubation at 32C.

Several reports (Borthwick et al., 1954; Carpita and Nabors, 1976; Scheibe and Lang, 1969) have shown that when seeds of cv. Grand Rapids are exposed to R and placed immediately at thermoinhibitory

Fig. 10 Germination of secondarily dormant seeds in response to KIN or GA_3.

Seeds of cv. Grand Rapids (II) were incubated for 4 days at 32C and transferred to fresh petri dishes containing distilled water, 100 ug/ml GA_3, or 10 ug/ml KIN. All samples were then exposed to R and transferred to the indicated temperatures. Each point represents the mean of 4 samples.

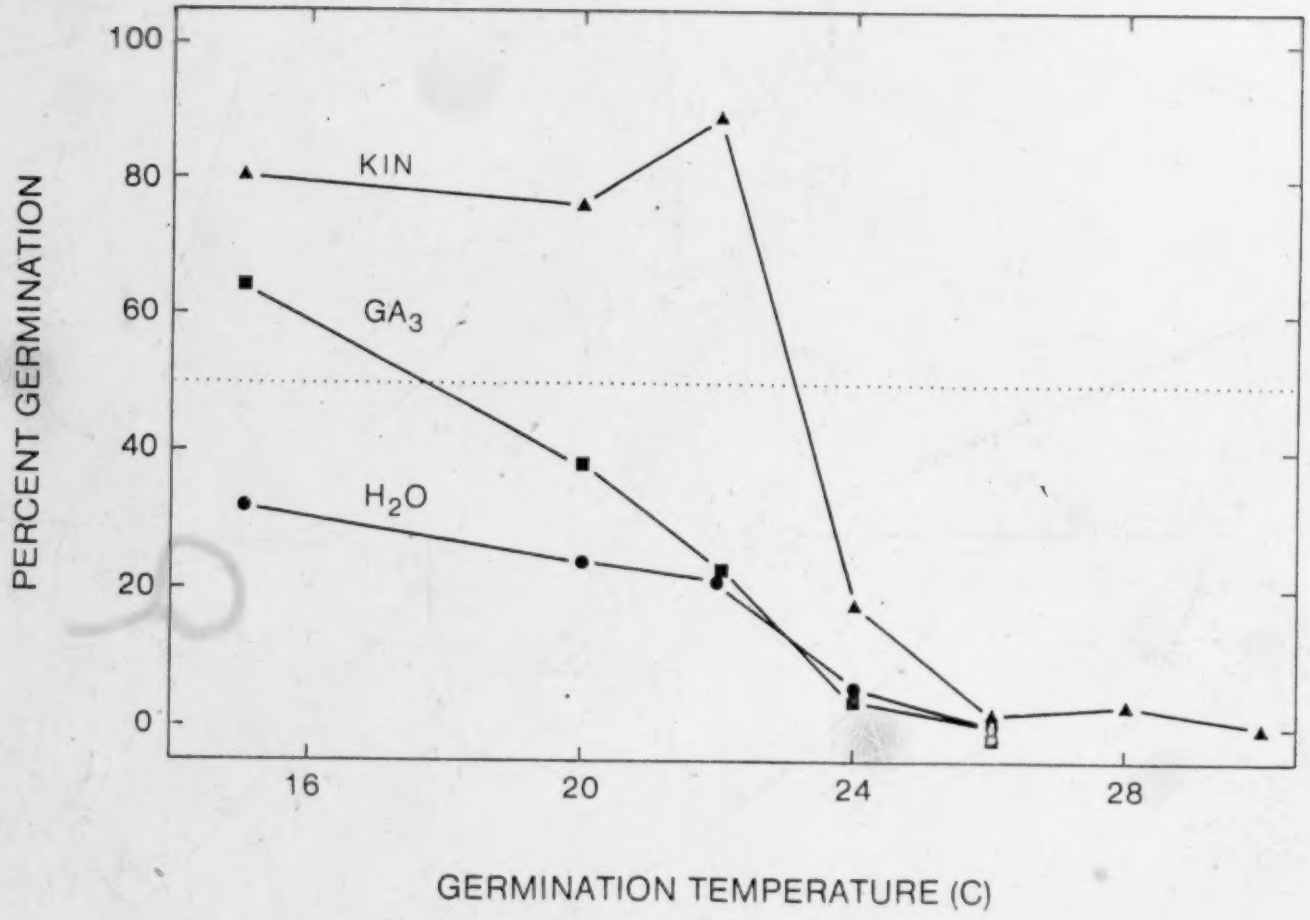
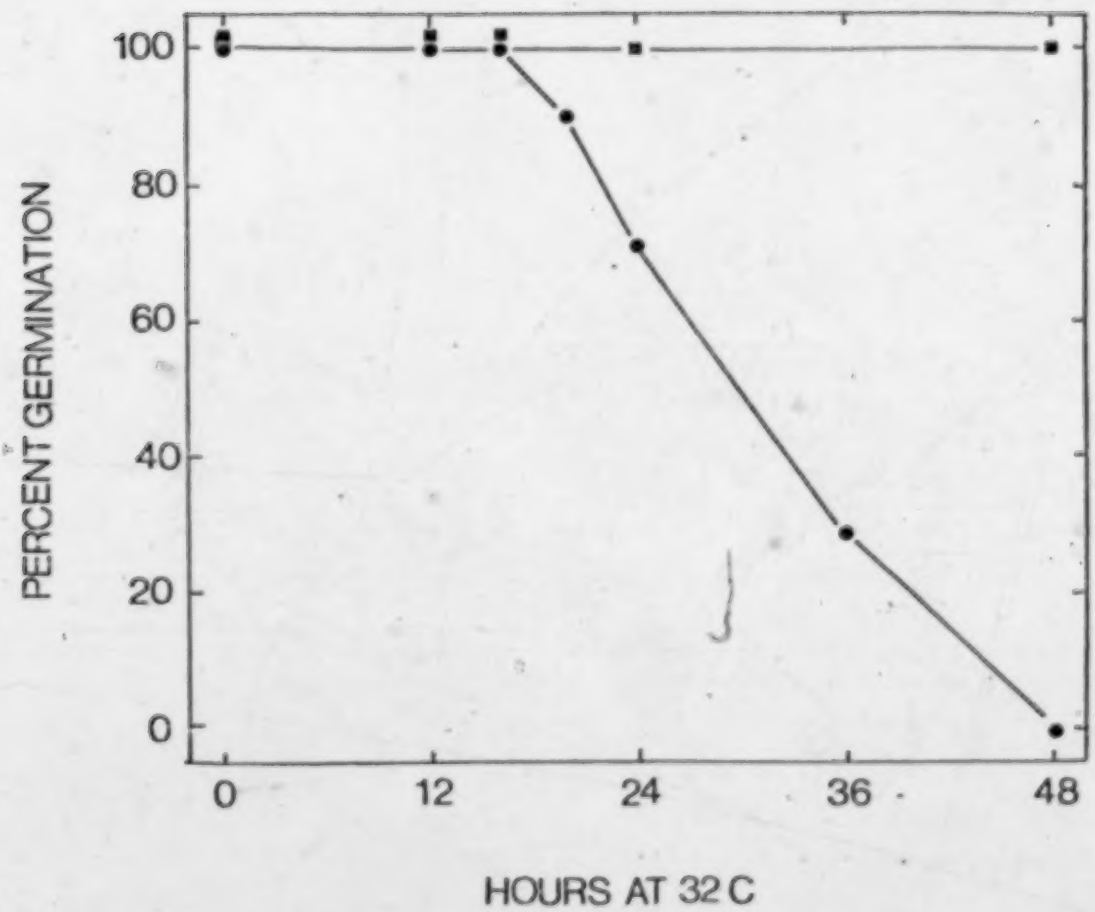

PERCENT GERMINATION
100
80
60
40
20
0
KIN
GA_3
H_2O
16
20
24
28
GERMINATION TEMPERATURE (C)

Fig. 11 Reversal of R promoted germination by incubation at 32C.

Seeds of cv. Grand Rapids (II) were imbibed one hour at 20C before R irradiation and transferred to 32C. After varying durations of 32C incubation the seeds were either transferred directly to 20C (●) or were exposed to R and transferred to 20C (■). Each point represents the mean of 4 samples.


PERCENT GERMINATION
100
80
60
40
20
0
0
12
24
36
48
HOURS AT 32 C

temperatures, the promotive effect of R on subsequent germination at 20C can be lost. Since germination at 20C can usually be restored by a second exposure to R, the decline in germination potential is thought to be related to the loss of Pfr.

A similar experiment was conducted with seeds of cv. Grand Rapids (II). The results shown in Fig. 11 indicate that the promotive effects of R were totally lost after 48 hours of high temperature incubation. However, a second exposure to R promoted full germination. These results indicate that Pfr is rapidly lost during incubation at high temperature.

3.11 Effect of light on the induction of secondary dormancy in lettuce.

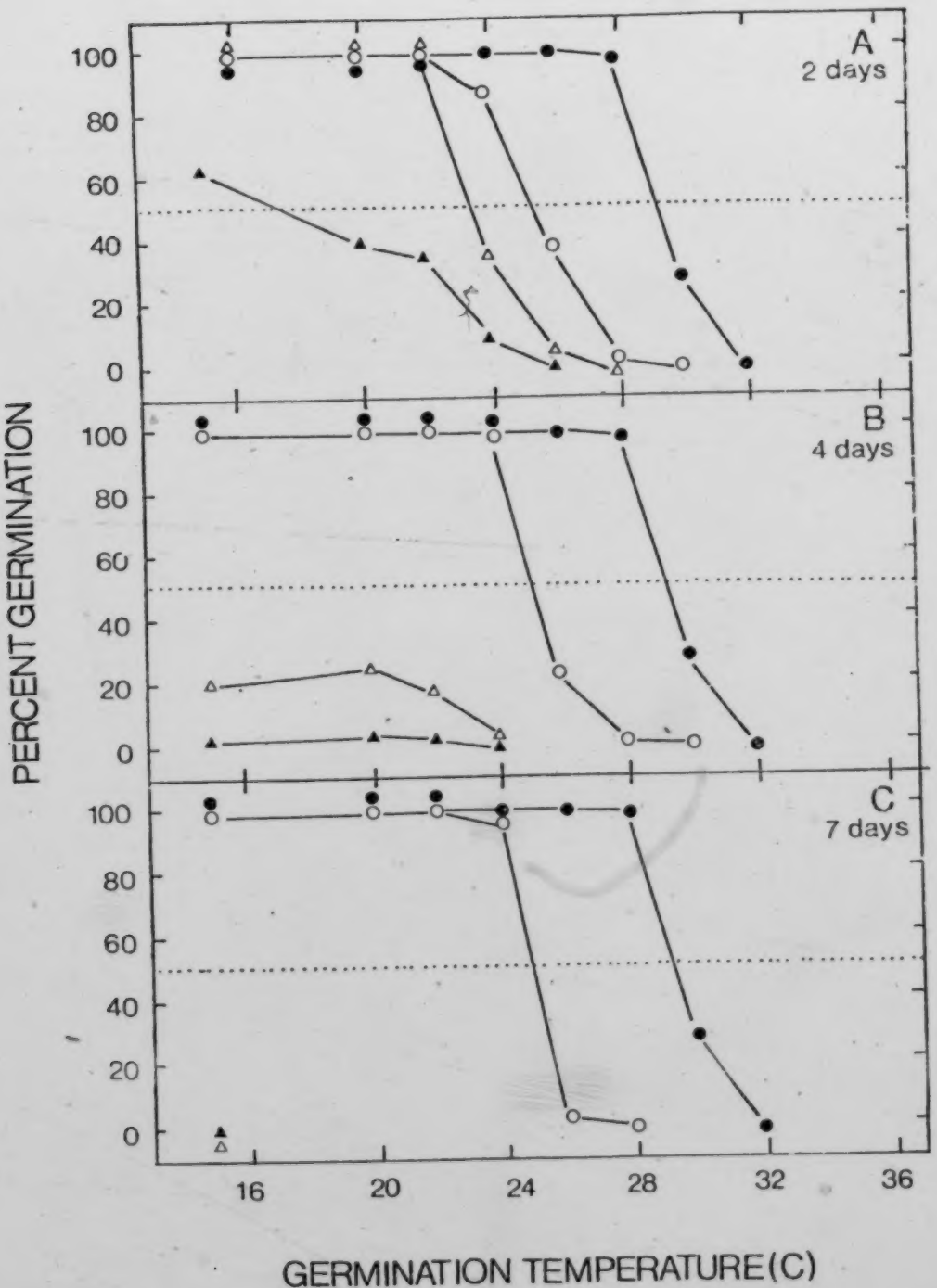
Vidaver and Hsiao (1975) found that seeds incubated at 30C eventually became secondarily dormant even if they were exposed to R prior to the 30C incubation. They concluded that the induction of secondary dormancy was not prevented by the presence of Pfr. However, the data of Fig. 11 indicate that Pfr is lost during high temperature incubation. Therefore, the experiments of Vidaver and Hsiao (1975) may not adequately test the effects of Pfr on the induction of secondary dormancy.

To re-examine this point, seeds were exposed to R at the beginning of a 32C incubation (Initial R) or were exposed to R repeatedly throughout the incubation period (Repeated R). It was assumed that repeated exposures to R would maintain phytochrome in the Pfr form during the course of the high temperature incubation.

The results shown in Fig. 12 indicate that the phytochrome status of the seed does influence the induction of secondary dormancy.

Fig. 12 Effect of light on the induction of secondary dormancy in lettuce.

Seeds of cv. Grand Rapids (II) were incubated at 32C for 2, 4 or 7 days under varying light conditions: Initial R - 5 min R one hour after the start of imbibition (△); Repeated R - 5 min R every 30 min throughout the incubation period (○); Dark (▲). At the end of the 32C incubation all seeds were exposed to R and transferred to the indicated temperatures. A control treatment (●) was imbibed one hour at 24C, exposed to R, and transferred to the indicated temperatures. Each point represents the mean of 4 samples.


A
2 days
B
4 days
C
7 days
PERCENT GERMINATION
100
80
60
40
20
0
16
20
24
28
32
36
GERMINATION TEMPERATURE (C)

Compared to the dark control, both Initial R and Repeated R treatments slowed the decline of the $UTCP_L$ (e.g. Fig. 12, 2d). However, longer incubations eventually imposed secondary dormancy on Initial R treated seeds. In contrast, secondary dormancy was not imposed on seeds receiving repeated exposures to R, even when the incubation period was extended to 7 days. These results suggest that Pfr (or its products) in some way "protects" the seeds against the induction of secondary dormancy. Initial R treated seeds eventually become dormant, presumably because of the loss of Pfr or its products at high temperature.

Although secondary dormancy was not imposed on seeds receiving repeated exposures to R, a considerable decline in $UTCP_L$ did occur, particularly within the first two days of high temperature incubation. During the next five days of incubation, little or no further decline occurred. This suggests that maintaining Pfr in the seed prevented the induction of secondary dormancy, rather than just delaying its appearance.

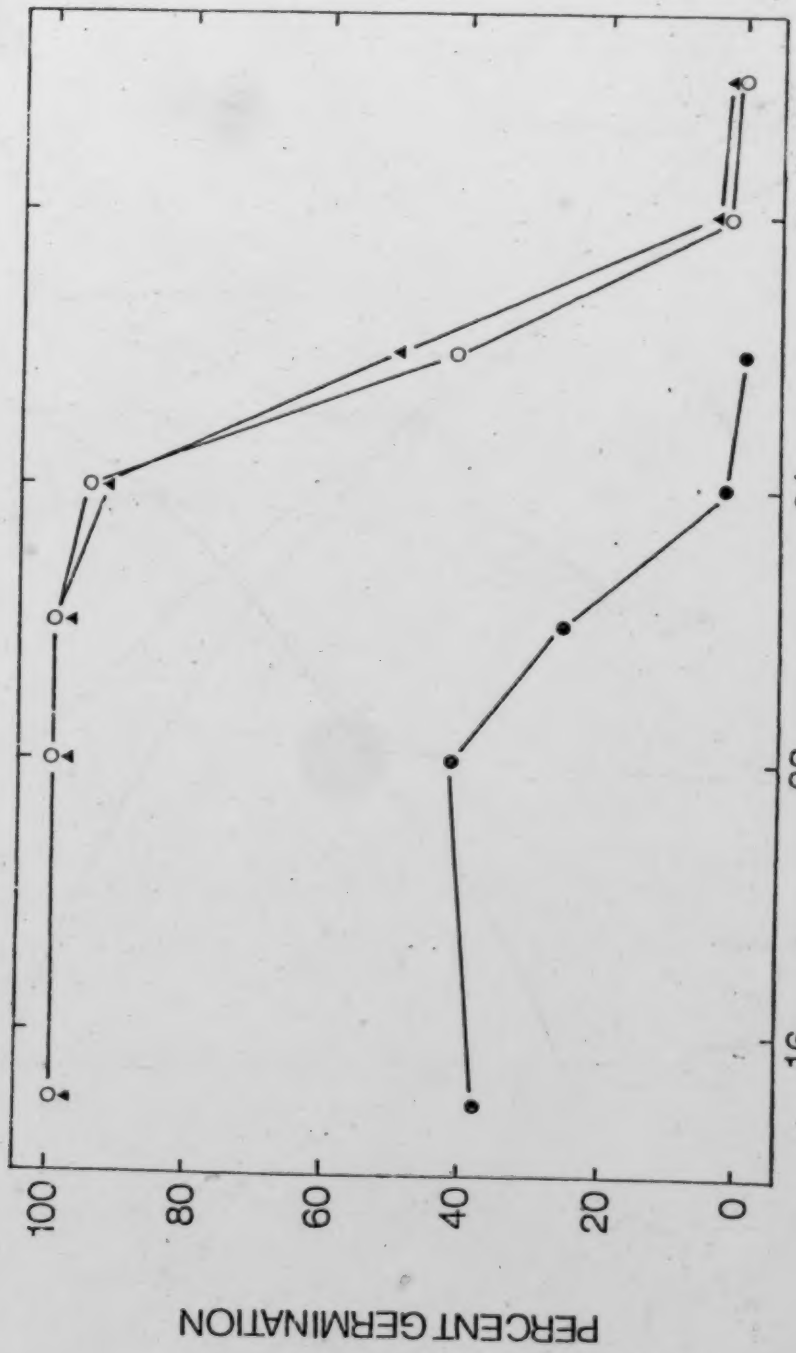
3.12 Effect of GA_3 on the induction of secondary dormancy in lettuce.

Gibberellic acid was effective in promoting the dark germination of cv. Grand Rapids (II). An experiment was devised to determine if GA_3 could also mimic the "protective" effect of R.

Seeds were imbibed in GA_3 or received repeated exposures to R during the course of a 4 day incubation at 32C (Fig. 13). However, it had been previously shown that $R+GA_3$ promotes the germination of secondarily dormant seeds (Fig. 10). Therefore, transferring seeds incubated in GA_3 to lower temperatures and exposing them to R, would be expected to cause a slight promotion in germination (i.e. compared

Fig. 13 Effect of GA_3 on the induction of secondary dormancy in lettuce.

Seeds of cv. Grand Rapids (II) were incubated for 4 days at 32C either in 100 ug/ml GA_3 (▲) or under Repeated R conditions (○). A control treatment (●) consisted of dark incubation in H_2O. Following 32C incubation all samples were exposed to R and transferred to fresh solutions of 100 ug/ml GA_3. Germination was determined at the indicated temperatures. Each point represents the mean of 3 samples.


PERCENT GERMINATION
0
20
40
60
80
100
16
20
24
28
GERMINATION TEMPERATURE(C)

to water controls). In addition, simply transferring the GA_3 incubated seeds to distilled water at the end of the incubation period, would not account for the GA_3 taken up within the seed. Therefore, to prevent differences in GA_3 levels among treatments, all samples were transferred to solutions of GA_3 at the end of the 32C incubation.

The results indicate that GA_3 is as effective as R in preventing the induction of secondary dormancy.

3.13 Escape from FR reversibility at 32C.

The data of Fig. 12 showed that if Pfr was maintained in the seed during a high temperature incubation, the induction of secondary dormancy was prevented. The following experiment was conducted to determine if the escape reactions of the phytochrome system proceeded during this period.

Seeds were incubated for 0.5, 1, 2 or 4 days at 32C under Repeated R conditions. At the end of the high temperature incubation the seeds were exposed to FR and transferred to germination temperatures. It was found that zero germination occurred at all temperatures (i.e. 15-30C), regardless of the length of the 32C incubation. Therefore, even after 4 days of incubation, these "protected" seeds required the presence of Pfr at low temperatures in order to germinate. This means that the escape reaction of the phytochrome system had not proceeded to completion during the 32C incubation.

While these data shed little light on the nature of the "protective" effect of Pfr, they do suggest that the inhibition of germination at high temperatures is related to a block in the phytochrome mediated germination pathway.

The literature concerning the effects of thermoinhibitory temperatures on the phytochrome system is equivocal. Scheibe and Lang (1969) found that in cv. Grand Rapids Pfr cannot function at temperatures above 32C. Ikuma and Thimann (1964) concluded that the inhibitory effects of high temperature on cv. Grand Rapids arose from a general inhibition of the post inductive phase (i.e. escape reactions) of germination. Both reports suggest that the inhibitory effects of high temperature are related to an effect on the phytochrome system itself.

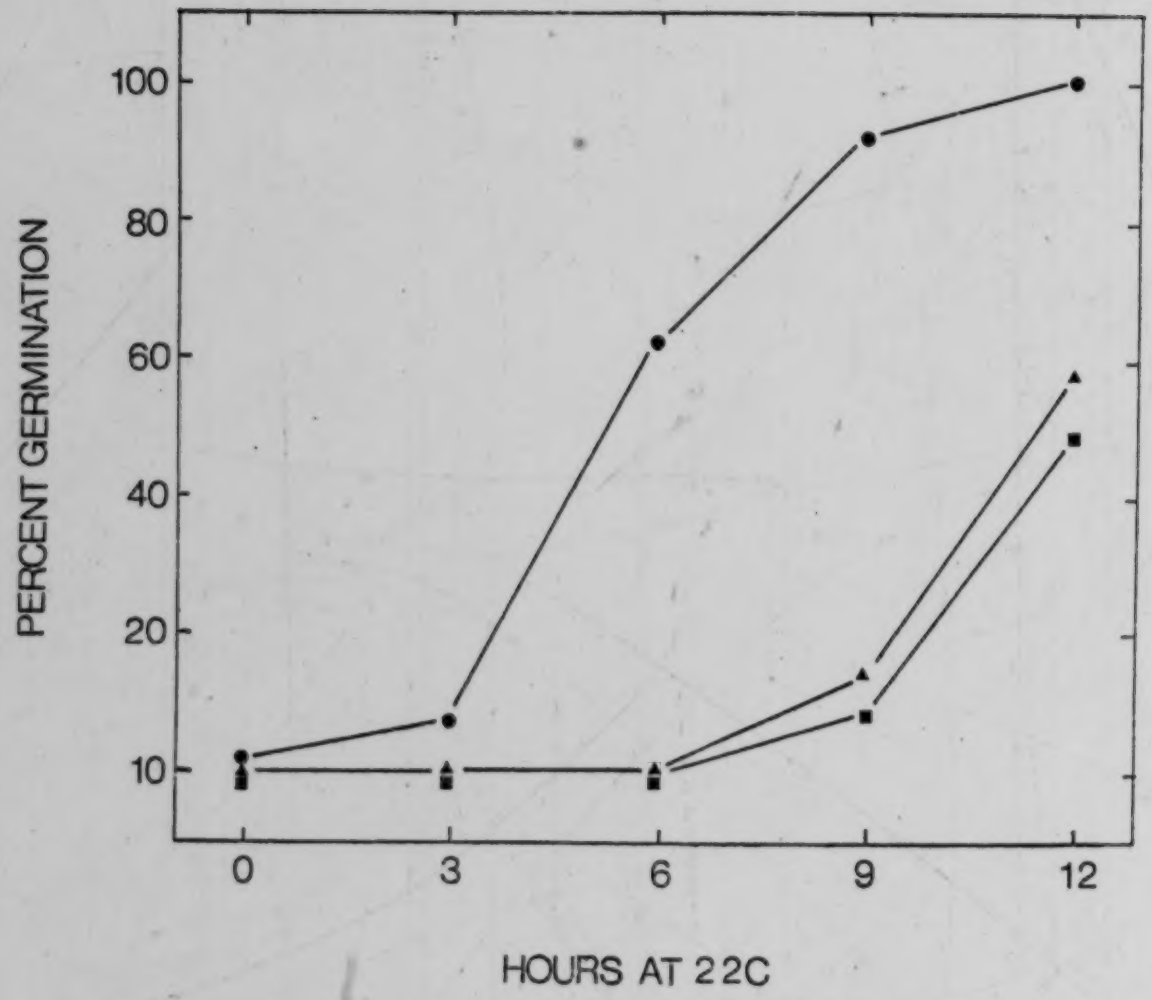
In contrast, Takeba and Matsubara (1976) found that in cv. New York, the escape reactions of the phytochrome system did proceed at thermoinhibitory temperatures. Since escape occurred, but germination did not, they concluded that thermoinhibition was not the result of a direct effect on the phytochrome system, but must result from some other block. Negm et al. (1973) showed that in cv. Great Lakes phytochrome was functional at 35C and that the escape reactions of the phytochrome system proceeded at this temperature. They concluded that the inhibition of germination at high temperature resulted from block(s) other than a direct inactivation of the phytochrome system.

3.14 Escape from the inhibitory effects of FR or high temperature.

The results of the previous experiment showed that in seeds of cv. Grand Rapids (II), thermoinhibitory temperatures blocked the escape reaction of the phytochrome system. If this was the only block to germination at high temperatures, then one might expect that seeds that had escaped the inhibitory effects of FR would also have escaped the inhibitory effects of high temperature.

Fig. 14 Escape from the inhibitory effects of FR or high temperatures.

Seeds of cv. Grand Rapids (II) were imbibed one hour at 22C, exposed to R, and incubated for varying periods at 22C. Following incubation at 22C the seeds were placed immediately at 32C (▲) or were exposed to FR and placed at 32 C (■) or 22C (●). Germination was determined 72 hours later. Each point represents the mean of 3 samples.


PERCENT GERMINATION
100
80
60
40
20
10
0
3
6
9
12
HOURS AT 22C

To test this hypothesis, seeds were exposed to R and incubated for varying periods at 22C before FR treatment or transfer to 32C. The results shown in Fig. 14 indicate that almost a complete loss of photoreversibility had occurred after 9 hours at 22C. However, escape from the inhibitory effects of high temperature had not occurred within this period. Radicle emergence occurred within 13 hours at 22C in this seed lot. Placing visibly germinated seeds at 32C did not prevent further growth of the radicle.

These results suggest that in cv. Grand Rapids high temperatures impose two blocks on germination. One block prevents escape from photoreversibility, while a second block prevents radicle emergence, but has no effect on radicle growth once emergence has occurred. It may also be speculated that the second block prevents germination in light-insensitive cultivars such as cv. Great Lakes.

3.15 Effect of osmoticum on the induction of secondary dormancy at 24C in lettuce.

Physiological preconditioning or priming of seeds has been conducted on a wide variety of species in an attempt to reduce the time between sowing and emergence (Heydecker, 1978). These treatments usually involve incubating seeds in solutions of high osmotic pressure, at temperatures of 10-20C (Khan, 1977). These treatments bring the seeds to the "brink" of germination but do not permit radicle emergence (Hegarty, 1978).

Although osmotic preconditioning improves the rate and uniformity of germination in lettuce cv. Grand Rapids, the percent emergence at high temperatures can be reduced by such treatments (Khan, 1977).

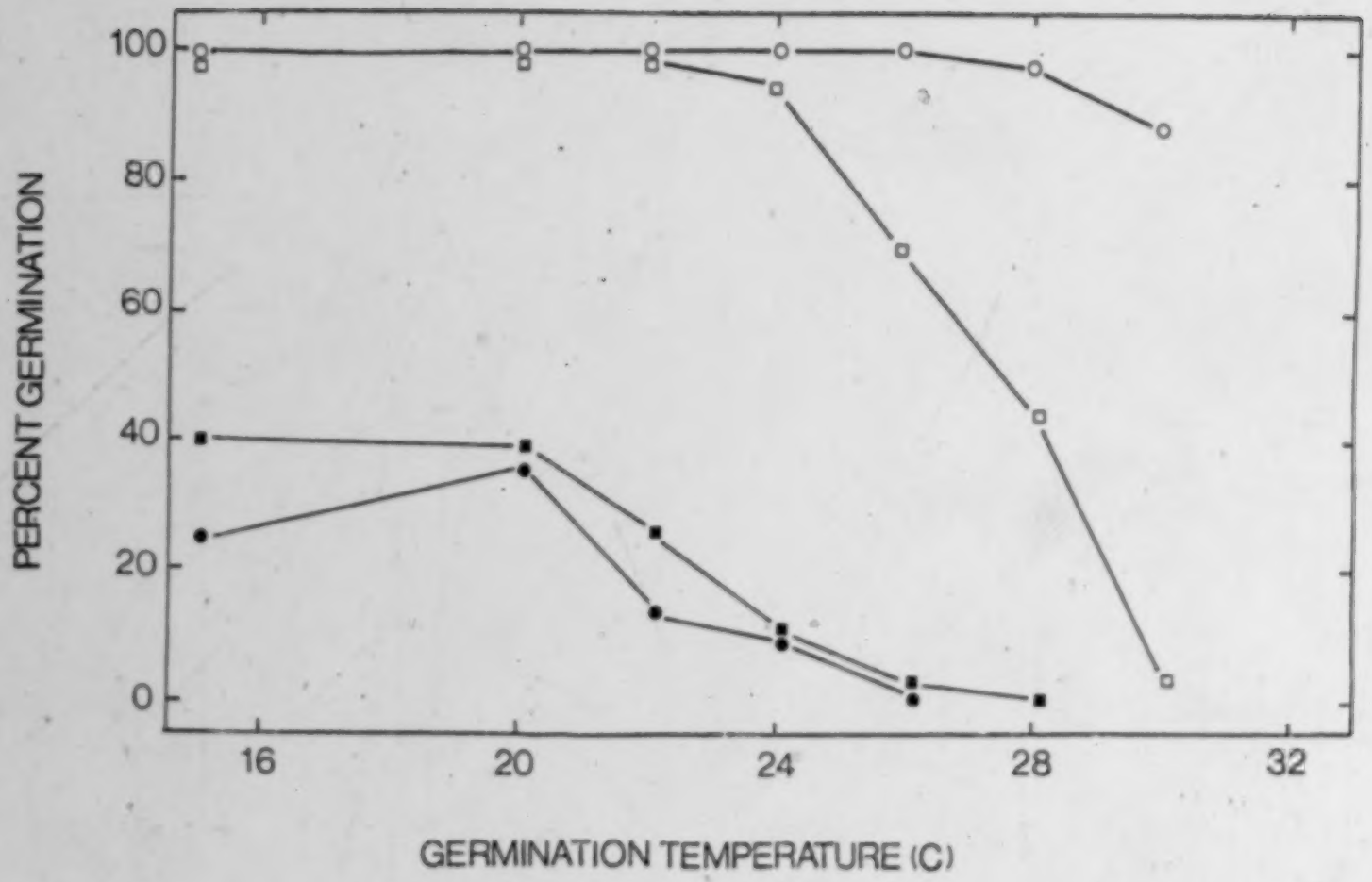
An experiment was conducted to determine if this reduced emergence is related to a decline in the $UTCP_L$. In a preliminary experiment it was found that 25% W/V PEG 6000 did not permit radicle emergence in seeds incubated under cool white fluorescent light for up to 10 days.

Seeds of cv. Grand Rapids (II) were incubated in 25% W/V PEG 6000 for four days before transfer to distilled water. The results shown in Fig. 15 indicate that secondary dormancy was induced in seeds receiving dark osmotic treatment as rapidly as in those incubated in water. Thus, reduced seed water content had little or no effect on the induction of secondary dormancy. Similarly, Duke (1978) found that incubation in PEG 6000 had no effect on the onset of secondary dormancy in Rumex crispus L.

Repeated R treatments were highly effective in preventing a decline in the $UTCP_L$. Initial R treatment was less effective. This may suggest that longer incubations would eventually impose secondary dormancy even in Initial R treated seeds. Kahn (1960) had previously shown that a light requirement could be induced in non photodormant seeds of cv. Grand Rapids by prolonged incubation in mannitol at 20C. Similarly, Khan and Karssen (1979) found that a secondary light requirement (i.e. an induced photodormancy) could be induced in seeds of cvs. Grand Rapids and Mesa 659 by prolonged incubation in PEG 6000. Continuous or daily R irradiation could prevent the induction of this photodormancy. Neither report mentions a reduction of germination in response to R after prolonged incubations.

Fig. 15 Effect of osmoticum on the induction of secondary dormancy at 24C in lettuce.

Seeds of cv. Grand Rapids (II) were incubated for 4 days at 24C, in 25% W/V PEG 6000, under varying light conditions i.e. Repeated R (○); Initial R (□); Dark (■). A dark control (●) was incubated in water. Following incubation at 24C all treatments were transferred to petri dishes containing distilled water, exposed to R, and placed at the indicated temperatures. Each point represents the mean of 3 samples.


PERCENT GERMINATION
100
80
60
40
20
0
16
20
24
28
32
GERMINATION TEMPERATURE (C)

3.16 Effect of seed transfer on the germination of heat treated lettuce seeds.

It was observed during the course of these investigations that transferring secondarily dormant seeds to fresh petri dishes containing distilled water caused a slight promotion of germination. This "transfer effect" was further investigated by incubating seeds of cv. Grand Rapids (I) at 35C for varying periods and transferring the seeds to 20 or 26C (Fig. 16).

Seed transfer promoted germination at 26C in seeds incubated for one to three days at 35C, however, it had little effect on germination at 26C, for seeds incubated longer than three days. In contrast, seed transfer was highly effective in promoting germination at 20C, even in seeds that had been incubated for eight days at 35C. These results suggest that the transfer effect is strongly temperature dependent.

3.17 Effect of seed transfers on the $UTCP_L$ of secondarily dormant lettuce seeds.

The effect of seed transfer was investigated over a wide range of temperatures (Fig. 17). Transfer of seeds incubated for six days at 35C promoted germination at all temperatures up to 22C but had little effect on germination at higher temperature. A sharply defined $UTCP_L$ was re-established at approximately 23C. This compares to a $UTCP_L$ of approximately 31C in fresh or untreated seeds (Fig. 6C, 0d).

Fig. 16 Effect of seed transfer on the germination of heat treated lettuce seeds.

Seeds of cv. Grand Rapids (I) were incubated at 35C for varying periods before R irradiation. The seeds were then transferred to either fresh petri dishes containing distilled water (O) or were left on their original dishes (●). Germination was determined after 72 hours at 20 or 26C. Each point represents the mean of 4 samples.

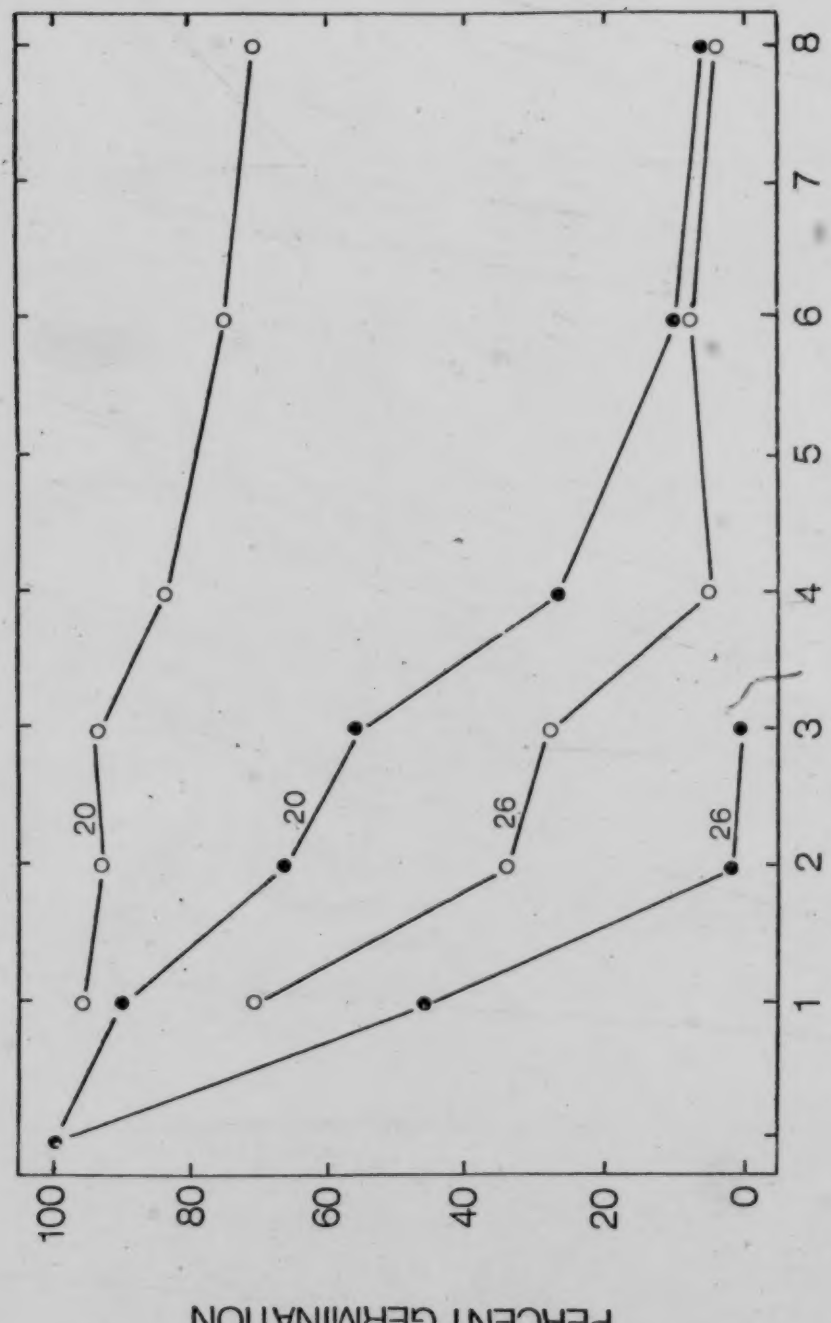
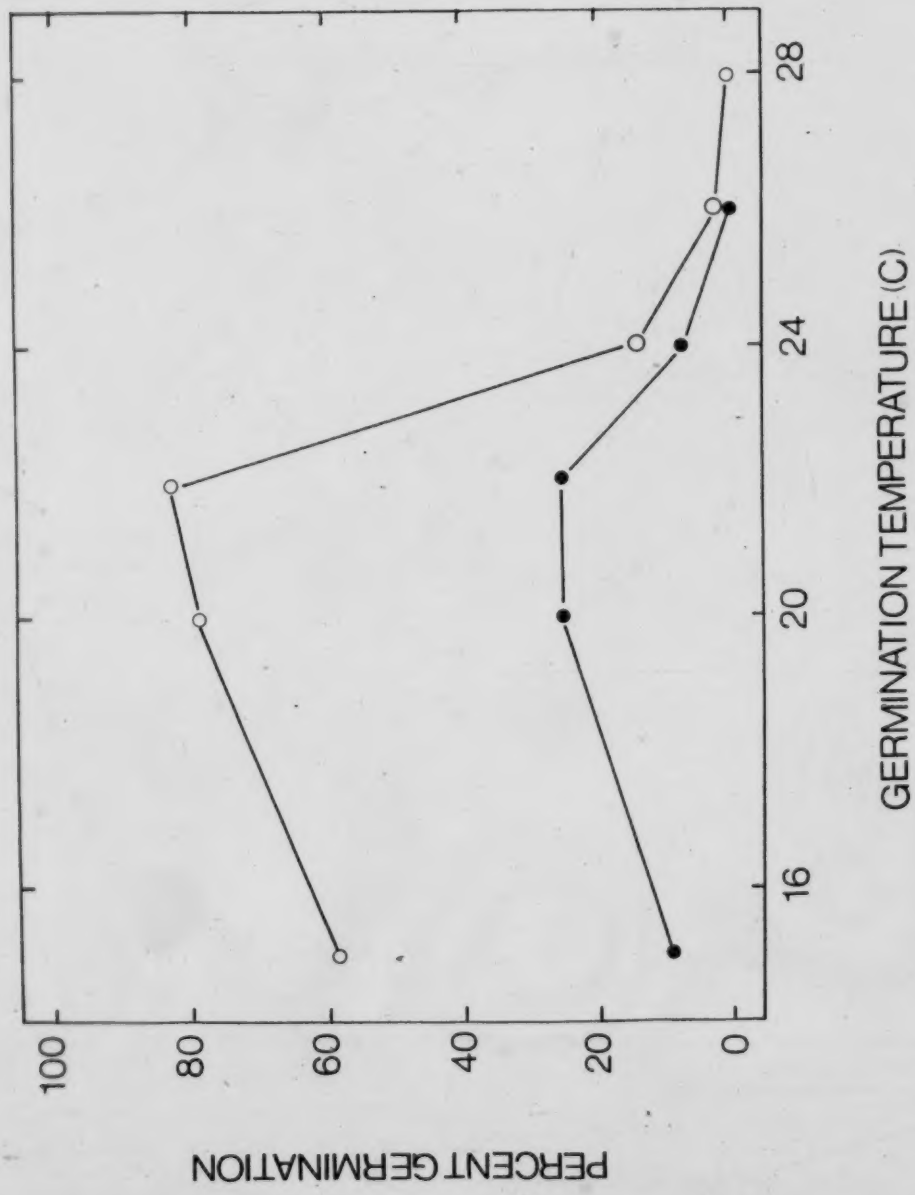

PERCENT GERMINATION
100
80
60
40
20
0
1
2
3
4
5
6
7
8
DAYS AT 35C
20
20
26
26

Fig. 17 Effect of seed transfer on the $UTCP_L$ of the secondarily dormant lettuce seeds.

The procedure used was the same as in Fig. 16. Seeds of cv. Grand Rapids (I) were made secondarily dormant by 6 days incubation at 35C. Transferred (O); Not transferred (●). Each point represents the mean of 4 samples.


PERCENT GERMINATION
100
80
60
40
20
0
16
20
24
28
GERMINATION TEMPERATURE (C)

3.18 Effect of seed transfer and movement of seeds on the germination of secondarily dormant lettuce seeds.

It is well known that damaging, removing or otherwise disturbing the seed covers can promote the germination of photodormant or thermoinhibited lettuce seeds (Borthwick and Robbins, 1928; Evanari and Neuman, 1953; Foard and Haber, 1966; Ikuma and Thimann, 1963). An experiment was done to determine whether the "transfer" effect was a result of a mechanical stimulation or removal of the seeds from the presence of an inhibitor in the medium.

The results in Table 2 clearly indicate that the transfer effect cannot be totally explained in terms of a mechanical effect. The data suggest the presence of an inhibitor in the germination media of secondarily dormant seeds.

3.19 Effect of "old" petri dishes on the germination of fresh seeds.

In this experiment fresh or untreated seeds were used to test for the presence of an inhibitor in the germination media of secondarily dormant seeds ("old" petri dishes). The germination tests were conducted at temperatures close to the $UTCP_L$ on the assumption that seeds would be most sensitive to "inhibitors" (Reynolds, 1975b) at these temperatures.

The results summarized in Table 3 do not indicate the presence of an inhibitor in the germination media. A variety of similar experiments conducted with fresh lettuce seeds also failed to provide any evidence for an inhibitor in the germination water of secondarily dormant seeds. These results could be explained by assuming that high temperature incubations sensitized seeds to some factor that was not

Table 2

Effect of seed transfer and movement of seeds on the germination of secondarily dormant lettuce seeds.

TREATMENT	PERCENT GERMINATION
Unmoved control	18±2
Removed and returned to original petri dishes	34±2
Moved within petri dishes	23±2
Transferred to new petri dishes	63±2

Seeds of cv. Grand Rapids (I) were made secondarily dormant by a 48 hour incubation at 35C. After manipulations of the seeds were complete, the seeds were exposed to R and transferred to 24C. Each value represents the mean of 10 samples.

Table 3

Effect of "old" petri dishes on the germination of fresh seeds.

	PERCENT GERMINATION	
Temperature (C)	H_2O	"Old" Dishes
30	41±4	37±4
28	91±2	90±4
26	96	94±2
24	95±1	93±2

"Old" petri dishes were prepared by incubating 500 seeds on each petri dish for 96 hours at 35C. Fresh seeds were imbibed one hour at 24C before R irradiation. Germination was determined after 24 hours at the indicated temperatures. Each value represents the mean of 4 samples.

normally inhibitory. However, it seems equally likely that some factor in the fresh petri plates could have promoted the germination of secondarily dormant seeds. This would explain why no inhibition occurred when fresh seeds were imbibed on "old" petri dishes. This latter possibility was not investigated.

Several previous studies have indicated that an inhibitor may leach out of photodormant or non dormant lettuce seeds into the germination media (Schuck, 1935; Sharples, 1978; Stout, 1941). It has been shown that the repeated germination of seeds in the same germination media progressively reduces the germination of cv. Grand Rapids (Schuck, 1935). Water extracts of cv. Grand Rapids but not of cv. New York have been shown to inhibit the germination of lettuce (Stout, 1941). It has recently been suggested that non dormant lettuce seeds excrete an inhibitor into the seed micro-environment (Sharples, 1978). It is thought that at low moisture potentials the inhibitor is unable to diffuse away rapidly, and therefore it affects germination. Increasing moisture potentials, or placing activated carbon in the germination media was found to increase germination. Concentrated extracts of absorbed substances eluted from the activated carbon were shown to inhibit germination. However, a recent report (Pecket and Al-Charchafchi, 1978) which involved seed transfer experiments similar to those presented here, stated that there was no evidence for the involvement of a leachable endogenous inhibitor in the germination of photodormant seeds.

Inhibitory substances leached into the germination media have also been linked to the induction of secondary dormancy (Speer and Tupper, 1975). It was found that partially purified and concentrated

extracts of the "germination water" of secondarily dormant seeds contained inhibitory substances, one of which was probably ABA. However, it was not demonstrated that the germination water taken directly from petri dishes (i.e. not concentrated) had any effect on germination.

The present data do not permit any firm conclusion on the nature of the transfer effect described here. However, the data do point out an important promotive effect on germination that must be taken into account during experiments with secondarily dormant seeds.

4.0 General Discussion

The present investigation has centered on the role that temperature plays in the induction and release of secondary dormancy in lettuce. The results presented here demonstrate that the induction of secondary dormancy in cv. Grand Rapids is related to a decline in the $UTCP_L$.

Some of the findings of the present work are consistent with the involvement of two distinct processes in the suppression of germination by prolonged incubations under non-germinating conditions.

1) In cv. Grand Rapids short incubations under non-germinating conditions reduced germination only in the vicinity of the $UTCP_L$ (i.e. they had an "inhibitory" effect). However, long incubations reduced germination at all temperatures (i.e. they had a "toxic" effect).

2) Incubation of seeds of cv. Grand Rapids (II) in GA_3 or under Repeated R conditions prevented the induction of secondary dormancy, but did not prevent a decline in the $UTCP_L$.

3) Incubation of seeds of cv. New York at 32C reduced their $UTCP_L$, but did not induce a secondary dormancy.

These observations may suggest that separate processes control the inhibitory and toxic stages of dormancy induction. However, the present data do not rule out the possibility that these toxic and inhibitory stages are the result of the same or similar processes, carried to different degrees of completion.

The nature of the processes that lead to secondary dormancy are largely unknown. Vidaver and Hsiao (1975) found that the induction

of secondary dormancy in cv. Grand Rapids required oxygen, and occurred whenever germination was suppressed for a sufficient length of time. The present results showed that the rate of dormancy induction was temperature dependent. However, dormancy was induced even in seeds incubated at relatively low temperatures.

Vidaver and Hsiao (1974) postulated that the induction of secondary dormancy in cv. Grand Rapids results from a blockage of a GA_3 sensitive dark germination pathway with a concomitant loss of gibberellin activity required for a light sensitive pathway. This hypothesis was based on results that indicated that R+GA_3 was highly effective in promoting the germination of secondarily dormant seeds. The experimental procedure involved transferring secondarily dormant seeds to petri dishes containing GA_3. However, no mention was made of transferring the water controls to fresh petri dishes also. Therefore, it is possible that a portion of the promotive effect obtained with R+GA_3 was related to the transfer effect reported in the present work. An experiment presented in this thesis (3·9) indicated that R+GA_3 was much less effective than R+KIN in promoting the termination of secondarily dormant seeds. Similar results were obtained by Speer and Tupper (1975). Therefore there is little reason to believe that gibberellins play a central role in the induction of secondary dormancy.

Speer and Tupper (1975) suggested that the induction of secondary dormancy could be related to the accumulation of an inhibitor, possibly ABA, either in the seed itself or in the germination medium. Although evidence from transfer experiments was inconclusive, the presence of an inhibitor in the germination media of secondarily

dormant seeds seems doubtful (3·19). However, it is interesting to note that the inhibitory and toxic stages of secondary dormancy induction can be duplicated with varying concentrations of ABA (Reynolds, 1975b). Nevertheless, the validity of the inhibitor theory of secondary dormancy cannot be judged until endogenous ABA levels are examined during the induction of secondary dormancy. Several reports (Berrie and Robertson, 1976; Braun and Khan, 1975; Robertson and Berrie, 1977) have shown that the inhibition of germination by high temperatures cannot be related to changes in total ABA or to changes in the levels of "free" or "bound" ABA. However, no attempt has been made to study changes in endogenous hormone levels that might occur during the induction of secondary dormancy. Thus any discussion of hormonal relationships during the induction of secondary dormancy is purely speculative.

A previous study (Vidaver and Hsiao, 1975) had indicated that the induction of secondary dormancy in cv. Grand Rapids was independent of the phytochrome status of the seed. The present results clearly indicate that maintaining Pfr in the seed during prolonged incubations prevents the induction of secondary dormancy. This suggests that the induction of secondary dormancy involves an effect on the phytochrome system itself. Other data also supports this conclusion. In lettuce cv. Grand Rapids (Ikuma and Thimann, 1964) Rumex crispus L. (Duke et al., 1977; Taylorson and Hendricks, 1973) or Chenopodium album L. (Karssen, 1970) prolonged dark incubations cause a decrease in photosensitivity. Eventually all three species become totally unresponsive to R or secondarily dormant.

The induction of secondary dormancy in R. crispus did not affect phytochrome transformations, or appear to involve a decrease in

total phytochrome. The present results (3·9) and other work (Speer et al., 1974; Speer and Tupper, 1975) indicate that Pfr is present in secondarily dormant seeds exposed to R. However, Pfr cannot function to promote germination unless a growth regulator such as KIN is supplied.

Taylorson and Hendricks (1973) attributed the secondary dormancy of R. crispus to order⇄disorder changes in cooperative structures or to phase transitions. It was suggested that secondary dormancy could be the result of changes in the organization of membrane components, protein configuration or lipid phase transitions. Duke et al. (1977) concluded that the decline in photosensitivity and eventual imposition of secondary dormancy in R. crispus was related to decreased levels of "X", the component with which Pfr interacts. It was suggested that the X factor is a membrane with varying affinity for Pfr. Karssen (1970) indicated that the induction of secondary dormancy in C. album is related to the phytochrome process which is reversed by brief far red irradiations.

The ability of Pfr or GA_3 to prevent the induction of secondary dormancy has not been previously reported. The mechanism of this "protective" effect cannot be determined from the data presented here. However, if the dormancy mechanism of lettuce is similar to that of R. crispus, then it seems reasonable to suggest that Pfr or GA_3 act by preventing the loss or disorganization of the X factor.

It is also tempting to link the induction of secondary dormancy in cv. Grand Rapids to the fact that escape from FR reversibility does not occur at high temperatures in this cultivar (Scheibe and Lang, 1969). In contrast, escape from FR reversibility does occur at high

temperatures in cv. Great Lakes (Negm et al., 1973). However, the present results indicate that high temperature incubations do not induce secondary dormancy in this cultivar. We initially thought that repeated exposures to R, or incubation in GA_3 might prevent the induction of secondary dormancy in cv. Grand Rapids by permitting escape to occur. However, a subsequent experiment showed that the protective effect of Pfr or GA_3 did not depend on completion of the phytochrome escape reaction.

The possibility that the escape reaction of the phytochrome system proceeds at high temperatures in light insensitive cultivars such as cv. Great Lakes, but not in cv. Grand Rapids has important implications. Short high temperature incubations can reduce dark germination levels in cv. Grand Rapids. Since exposure to R can restore germination, this induced photodormancy has been attributed to the thermal reversion of Pfr to Pr (Guttermann et al., 1972; Scheibe and Lang, 1965). It has also been found that high temperature incubations can induce photodormancy in cv. Great Lakes (Borthwick et al., 1952; 1954; Toole, 1959; 1961). However, if escape from FR reversibility occurs at high temperatures in cv. Great Lakes, then the induction of photodormancy in this cultivar must involve the loss of the products of the phytochrome system, as well as Pfr. Thus the induction of photodormancy in cvs. Great Lakes and Grand Rapids may not involve identical processes.

There is some evidence to support this hypothesis. In light insensitive cultivars such as Great Lakes or Cobham Green, the induction of photodormancy involves a decline in the $UTCP_D$ (Heydecker and Joshua, 1976; 1977). However, even after very prolonged high temperature incubations, full germination in the dark can still be obtained at low temperatures (i.e. 15C). In contrast, high temperature incubations

rapidly reduced the dark germination of cv. Grand Rapids at all temperatures (3·3). These results suggest that the induction of photodormancy in cv. Grand Rapids is analagous to the induction of secondary dormancy. That is, germination is suppressed at all temperatures. In contrast, the induction of photodormancy in light insensitive cultivars, seems to involve only a decline in the $UTCP_D$.

High temperatures (i.e. above the $UTCP_L$) prevent the germination of both light sensitive and light insensitive cultivars. Based on results obtained with seeds of cv. New York, Takeba and Matsubara (1976) have suggested that the germination of lettuce is controlled by a thermolabile factor. The thermolabile factor is thought to be deactivated at high temperatures, but can be reactivated at low temperatures. A stationary critical temperature for activation-deactivation was implied. However, it was assumed that activation was not identical to germination because fresh seeds could germinate at 25C but reactivation of heat treated seeds could not occur at this temperature.

These conclusions were based on the well known fact that imbibing seeds under conditions suitable for germination will eventually permit them to germinate even after transfer to thermoinhibitory temperatures (Berrie, 1966; Gray, 1977). Takeba and Matsubara found that if seeds were first incubated at high temperatures (e.g. 30C), the subsequent period at low temperatures needed to permit germination at 30C was increased (i.e. compared to seeds not receiving a 30C pretreatment). Progressively longer periods at high temperatures (i.e. deactivation) increased the length of low temperature incubation needed for reactivation.

In assessment of their work, several points suggest that the thermolabile factor does not control absolute germination levels. Instead, activation or deactivation of the thermolabile factor appears to be the result of imbibing seeds under germinating or non-germinating conditions. However, the thermolabile factor may be involved in controlling the rate of germination.

The ability of a particular temperature to permit reactivation was determined by incubating heat treated seeds at various temperatures (e.g. 15 or 25C) and then determining the germination that could be obtained at 30C. However, it is reasonable to assume that reactivation can only occur at a temperature that permits germination (i.e. if a seed cannot germinate at 25C, then incubating it at 25C will never enable it to germinate at 30C). Thus the critical temperature for activation-deactivation must be the UTCP.

Data presented here (3·4) showed that prolonged high temperature incubations of cv. New York reduced its $UTCP_L$. This would explain why Takeba and Matsubara found that germination of fresh seeds could occur at 25C, but reactivation of heat treated seeds could not.

Prolonged high temperature incubations also reduce the rate of lettuce seed germination at lower temperatures (Carpita and Nabors, 1976; Gray, 1977). This suggests that the deactivation of some thermolabile factor affects the rate of germination.

The mechanism that prevents germination at temperatures above the UTCP is somewhat controversial. Because escape from FR reversibility occurs at high temperature in cv. Great Lakes, Negm et al. (1973) concluded that thermoinhibition cannot be the result of a direct effect on the phytochrome system, but must result from some other block.

A similar conclusion was reached for seeds of cv. New York (Takeba and Matsubara, 1976). In contrast results obtained here with cv. Grand Rapids suggest that escape from FR reversibility does not occur at high temperatures. Therefore, at least one effect of high temperatures is to block the phytochrome mediated germination pathway. This conclusion is supported by the results of Scheibe and Lang (1969) and Ikuma and Thimann (1964). At the present moment there is no obvious explanation for these conflicting viewpoints.

The nature of the biochemical mechanism that underlies the UTCP is unknown. Because a very large change in germination levels can occur with a temperature shift of only 1 or 2°C, Reynolds (1973) has suggested that some type of trigger mechanism may be involved, as opposed to changes in the reaction rates of some critical process (Berrie, 1966). The order⇆disorder transitions proposed by Taylorson and Hendricks (1973) are ideal candidates for this trigger mechanism. Order disorder transitions in cooperative states are said to have a high temperature dependence over some small temperature interval (Taylorson and Hendricks, 1973). Thus, the inhibition of germination at temperatures above the UTCP would result from disorder in some component critical to germination, possibly the X factor.

This hypothesis implies that the mechanism preventing the germination of thermoinhibited seeds is similar to that preventing the germination of secondarily dormant seeds. There is some evidence to support this view. For example, R alone was unable to promote the germination of secondarily dormant or thermoinhibited seeds (Speer et al., 1974; Vidaver and Hsiao, 1974). However, R+KIN or R+C_2H_4 promoted the germination of both thermoinhibited and secondarily dormant

seeds (Burdett, 1972b; Keys et al., 1975; Negm et al., 1973; Sharples, 1973; Speer et al., 1974; Speer and Tupper, 1975). In addition, results presented in this thesis indicate that the induction of secondary dormancy is related to a decline in the $UTCP_L$. In other words, prolonged incubations under non-germinating conditions lower the critical temperature of the trigger mechanism ($UTCP_L$), until eventually the seeds become thermoinhibited at all temperatures (i.e. secondarily dormant).

Reynolds and Thompson (1973) have shown that the position of the $UTCP_L$ on a temperature scale can be affected by the application of growth regulators such as GA_3, KIN or ABA. It seems reasonable to assume that a seeds complement of endogenous growth regulators will, by acting upon the trigger mechanism, also establish an $UTCP_L$ above which germination will not occur. Therefore it is not necessary to postulate changes in endogenous hormone levels (eg ABA) to account for the onset of thermoinhibition. However, during prolonged incubations under non-germinating conditions, a shift in the $UTCP_L$ does occur. To account for this decline in $UTCP_L$ one could postulate various hormonal changes such as an increase in inhibitors or a decrease in cytokinins. However, as was mentioned previously, no attempt has been made to investigate changes in endogenous hormone levels that occur during the induction of secondary dormancy.

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